


OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361Page A
2UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

DATE: May 13, 2003

MEMORANDUM

SUBJECT: **Acifluorfen** (Tackle/Blazer): Review of 3 mechanism studies:

- 1) The induction of the number and size of hepatic peroxisomes in mice following 4 week dietary feeding with acifluorfen (MRID 45693401),
- 2) S-phase response study in the liver of mice following 3 days, 1 week, and 2 weeks feeding with acifluorfen (MRID 45803601) and
- 3) Enzyme induction study in the liver of mice following 4 weeks feeding with acifluorfen (MRID 45793901).

DP Barcode: D283890, 283891, 286773, 286776
PC Code: 114402
TXR No. 0051328TO: Christina Scheltema
Review Branch 3
Special Review and Reregistration Division (7508W)FROM: Paul Chin 
Reregistration Branch 1
Health Effects Division (7509C)THRU: Whang Phang, Senior Scientist 
Reregistration Branch 1
Health Effects Division (7509C)

The registrant, BASF, submitted 3 main mechanism studies with acifluorfen (MRIDs 45693401, 45803601, and 45793901) and 3 studies with positive control (MRIDs 45686501, 45686502 and 45693402). One main study (MRID 45693401) was reviewed with the 3 positive control studies by the contractor, Oak Ridge National Laboratory, and the DER was appropriately modified to reflect the current policy and guidelines of the Agency. The other two main studies (MRID45803601 and 45793901) were reviewed by HED. The DERs for these studies are attached to this memorandum. The conclusion of each study including the citation and MRID number is presented below.

Main studies

Mellert, W., Kaufmann, W., van Ravenzwaay, B. (2002). Blazer technical (purity 46.1%): peroxisome proliferation study in B6C3F2 mice - administration in the diet for 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Project No. 99C0287/98154, BASF Doc. No. 2002/1005544. May 7, 2002. MRID 45693401. Unpublished.

Conclusion

The oral administration of Blazer Technical (46.1% acifluorfen-sodium) to mice for 4 weeks induced a dose-related increase in the number, size, and area of hepatic peroxisomes in the liver.

Mellert, W., Kaufmann, W., van Ravenzwaay, B. (2002). Blazer Technical: S-Phase Response Study in the Liver of B6C3F1 Mice - Administration in the Diet for 3 Days, 1 Week and 2 Weeks, conducted in BASF's Aktiengesellschaft Experimental Toxicology and Ecology Laboratory, Ludwigshafen/Rhine (GERMANY). BASF Registration Document No. 2002/1011947, Project No. 99CO287/98153, dated October 18, 2002. MRID 45803601. Unpublished.

Conclusion

The oral administration of Blazer Technical (46.1% acifluorfen-sodium) to mice for 2 weeks produced a dose-dependent and significant induction of bromodeoxyuridine (BrdU) labeling in the liver. The most pronounced effect for each of these three parameters (↑ liver weight, ↑ liver hypertrophy and ↑ BrdU labeling) for both sexes was seen after 1 week of treatment.

Mellert, W., Deckardt, K., Beimborn, D. and van Ravenzwaay, B. (2002). Blazer Technical: Enzyme Induction Study in the Liver of B6C3F1 Mice - Oral Administration in the Diet for 4 Weeks, performed at the BASF Aktiengesellschaft Experimental Toxicology and Ecology, Ludwigshafen/Rhine (GERMANY). BASF Registration Document No. 2002/1011353, (Project No.: 99CO287/98157), September 3, 2002. MRID 45793901. Unpublished.

Conclusion

The oral administration of Blazer Technical (46.1% acifluorfen-sodium) to mice for 4 weeks produced dose-dependent increase in cyanide-insensitive palmitoyl-CoA-oxidation (PALCoA) in the liver.

Positive control studies

Following positive control studies were conducted to confirm the sensitivity and reliability of the test system to detect peroxisome proliferation. The responses obtained with positive control studies adequately demonstrated the sensitivity of the test system.

Mellert, W., Kaufmann, W., van Ravenzwaay, B. (2001). **Positive control study** for support of peroxisome proliferation: DINP - peroxisome proliferation study in B6C3F1 mice - administration in the diet for 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Project No. 99C0266/00014, BASF Doc. No. 2001/5003598. January 23, 2001. MRID 45686501. Unpublished.

Mellert, W., Deckardt, K., Kaufmann, W., van Ravenzwaay, B. (2001). **Positive control study** for support of peroxisome proliferation: DINP - **enzyme induction study** in B6C3F1 mice administration in the diet for 1 and 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Project No. 99C0266/00018, BASF Doc. No. 2001/5003599. May 27, 2001. MRID 45686502. Unpublished.

Mellert, W., Kaufmann, W., van Ravenzwaay, B. (2001). **Positive control study** for support of peroxisome proliferation: DINP - **enzyme induction study** in B6C3F1 mice administration in the diet for 1 and 4 weeks: **Amendment** to BASF Reg. Doc. 2001/5003599: **Analysis of S-phase response**. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Project No. 99C0266/00018, BASF Doc. No. 2001/5003600. November 22, 2001. MRID 45693402. Unpublished.

B

DATA EVALUATION RECORD**ACIFLUORFEN/114402
NONGUIDELINE****STUDY TYPE: PEROXISOME PROLIFERATION STUDY - MICE
MRID 45693401 (MAIN STUDY)
MRIDs 45686501, 45686502, and 45693402 (POSITIVE CONTROL STUDIES)**

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 02-67

Primary Reviewer:

H. Tim Borges, Ph.D., MT(ASCP), D.A.B.T.Signature: *HT Borges*Date: OCT 29 2002

Secondary Reviewers:

Carol Forsyth, Ph.D., D.A.B.T.Signature: *Carol Forsyth*Date: OCT 29 2002Robert H. Ross, M.S., Group LeaderSignature: *Robert H. Ross*Date: OCT 29 2002

Quality Assurance:

Eric Lewis, M.S.Signature: *Eric B. Lewis*Date: OCT 29 2002

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

10/30/02

B. M. Clark

ACIFLUORFEN/114402

Nonguideline

EPA Reviewer: P. Chin, Ph.D.

Signature: Paul Chin

Reregistration Branch 1, Health Effects Division (7509C)

Date 3/13/03

Secondary reviewer, Whang Phang, Ph.D.

Signature: Whang Phang

Reregistration Branch 1, Health Effects Division (7509C)

Date 3/13/03

EPA Work Assignment Manager: PV Shah, Ph.D.

Signature: P.V. ShahRegistration Action Branch 1, Health Effects Division (7509C) Date 3/13/03**DATA EVALUATION RECORD****TRX#:** ~~0050846 & 0050847~~
0051328**STUDY TYPE:** 4-week Peroxisome Proliferation Study - Mice**PC CODE:** 114402**DP BARCODE:** D283890 & 283891**SUBMISSION NO.:** S617656-617657**TEST MATERIAL (PURITY):** Blazer Technical (46.1% a.i.)**SYNONYMS:** Blazer 2L; Acifluorfen, sodium; Tackle 2AS; sodium 5-((2-chloro-alpha, alpha, alpha-trifluoro-p-tolyl)oxy)-2-nitrobenzoate**CITATION:**

Mellert, W., Kaufmann, W., van Ravenzwaay, B. (2002). **Blazer technical** (purity 46.1%): peroxisome proliferation study in B6C3F2 mice - administration in the diet for 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Project No. 99C0287/98154, BASF Doc. No. 2002/1005544. May 7, 2002. MRID 45693401. Unpublished.

Mellert, W., Kaufmann, W., van Ravenzwaay, B. (2001). **Positive control study** for support of peroxisome proliferation: DINP - peroxisome proliferation study in B6C3F1 mice - administration in the diet for 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Project No. 99C0266/00014, BASF Doc. No. 2001/5003598. January 23, 2001. MRID 45686501. Unpublished.

Mellert, W., Deckardt, K., Kaufmann, W., van Ravenzwaay, B. (2001). **Positive control study** for support of peroxisome proliferation: DINP - **enzyme induction study** in B6C3F1 mice administration in the diet for 1 and 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Project No. 99C0266/00018, BASF Doc. No. 2001/5003599. May 27, 2001. MRID 45686502. Unpublished.

Mellert, W., Kaufmann, W., van Ravenzwaay, B. (2001). **Positive control study** for support of peroxisome proliferation: DINP - **enzyme induction study** in B6C3F1 mice administration in the diet for 1 and 4 weeks: **Amendment** to BASF Reg. Doc. 2001/5003599: **Analysis of S-phase response**. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056

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Ludwigshafen/Rhein, Germany. Project No. 99C0266/00018, BASF Doc. No. 2001/5003600. November 22, 2001. MRID 45693402. Unpublished.

SPONSOR: BASF Corp., Agricultural Products Group, P.O. Box 13528, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY:

The effects of 4-week dietary administration of Blazer Technical (Lot # 01501L300, purity 46%) on the induction of the number and size of hepatic peroxisomes in male and female B6C3F1 mice (5/sex/dose) was investigated (MRID 45693401). The administered concentrations were 0, 350, 1735, or 5210 ppm (*i.e.*: 160, 800 and 2400 ppm of the active ingredient). Mean daily intakes of the test substance were 0, 92/139, 484/644, and 1346/1939 mg/kg/day, [males/females], respectively and mean **daily intakes of the a.i.** were 42/64, 223/296 and 619/892 mg/kg, [males/females], respectively. In addition, one and 4-week positive control study on the peroxisome proliferator diisononyl phthalate (DINP) was conducted on B6C3F1 mice (5/sex/dose or 8/sex/dose). The test animals were fed diets containing 0, 500, 1500, 4000, or 8000 ppm. DINP (equivalent to 0, 117, 350, 913, and 1860 mg/kg/day in males and 0, 168, 546, 1272, and 2807 mg/kg/day in females, respectively) (MRIDs 45686501, 45686502, and 45693402).

DEFINITIVE STUDY

Blazer Technical induced a slight to moderate increase in the number of peroxisomes within the centrilobular region of the high-dose group of both sexes observable by light microscopy. No changes were found in male and female mice of lower dose groups. By electron microscopy, treatment with Blazer Technical induced a dose-related increase in the number, size, and area of hepatic peroxisomes in mice treated with ≥ 350 ppm test material, thereby suggesting that **Blazer Technical is a weak peroxisome proliferator.**

At 350 ppm, only slight increase of size 1 peroxisome (up to 1.5 fold; $p < 0.05$) in males and females were observed.

At 1735 ppm, significant increase in number of peroxisomes (2-6 fold; $p < 0.01$) in both sexes were observed. In both sexes of mice, dose-related significant increase ($p < 0.01$) in total peroxisomal area was apparent (2.7 fold increases in males and 2.2 fold increases in females).

At 5210 ppm, significant increase in number of peroxisomes in males (3-23 fold; $p < 0.01$) and females (1.5-35 fold; $p < 0.01$) were observed. In both sexes of mice, dose-related significant increase ($p < 0.01$) in total peroxisomal area was apparent (7.2 fold increases in males and 8.3 fold increases in females).

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The study investigating the induction of peroxisomes in the liver of treated male and female by Blazer technical is **Acceptable/Nonguideline** and demonstrates that **the test material is a weak peroxisome proliferator**, satisfying the study's intent.

POSITIVE CONTROL STUDIES

Examination by electron microscopy showed that DINP treatment for 4 weeks significantly increased the size, number, and cytoplasmic area of peroxisomes in male mice treated with ≥ 500 ppm and female mice treated with ≥ 1500 ppm. Particularly increased were the number of microperoxisomes in mice treated with ≤ 4000 ppm DINP. There were also a dose-related increase in cytoplasmic volume of male and female mice treated with ≥ 1500 ppm positive control (**MRID 45686501**).

Dose-dependent increases of cyanide-insensitive palmitoyl-CoA oxidation were found within mice treated with ≥ 500 ppm DINP and female mice treated with ≥ 1500 ppm for 4 weeks. The increases of palmitoyl-CoA oxidation were consistent with the dose-related increase of the number and size of peroxisomes in treated mice (**MRID 45686502**). Significant increases of liver weight in male and female mice were found following one week of treatment with 1500 ppm (females only), 4000 ppm, and 8000 ppm DINP and after 4 weeks of treatment with ≥ 1500 ppm. The dose-dependent increases are consistent with centrilobular hypertrophy and peroxisome proliferation.

After one week of treatment with DINP, the **S-phase response** was greater than controls in all treatment groups. After four weeks of DINP treatment, only male mice in the 500 and 4000 ppm groups showed more mitotic figures than control mice. In the remaining male and all female groups, mitotic figures were within the range of controls. Signs of apoptotic cell death were rare in control and DINP-treated groups (**MRID 45693402**).

The studies investigating the positive control DINP and its effects on peroxisome proliferation in male and female mice are **Acceptable/Nonguideline** and also satisfies the intent of the study.

COMPLIANCE: Signed and dated Quality Assurance, GLP, and Confidentiality statements were provided. A Flagging statement was not provided.

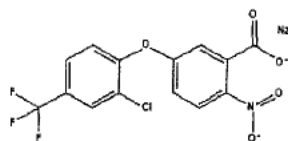
I. MATERIALS AND METHODS:

A. MATERIALS:

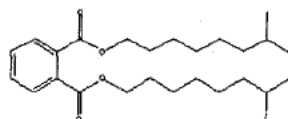
1. Test material:	Blazer Technical
Description:	Yellow-brown liquid
Lot/Batch #:	01501L300
Storage	Room temperature
Purity:	46% (44.9% by HPLC assay)

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Compound 2 years
Stability:
CAS # for 62476-59-9
TGAI:
Structure:



Positive Control: Diisononyl phthalate (DINP)
Description: Colorless liquid
Lot/Batch #: 9116SB34
Storage Room temperature (not otherwise specified)
Purity: 99.9%
Compound Stable
Stability:
CAS # for 68515-48-0
TGAI:
Structure:



2. Vehicle: Diet

3. Test animals (Definitive Study)

Species: Mice
Strain: B6C3F1
Age/weight at study initiation: 77 ± 2 days, male - 26.2-28.7 g; female 21.5-24.1 g
Source: Charles River, Sulzfeld, Germany
Housing: Individually in Macrolon cages
Diet: Maintenance diet, Provimi KLIBA SA, Kaiseraugst, Switzerland, *ad libitum*
Water: Tap water, *ad libitum*
Environmental conditions: 20-24°C
Temperature 30-70%
Humidity 12 hour light/dark
Photoperiod
Acclimation period: 6-7 days

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Test animals (Positive Control Studies)

Species: Mice
Strain: B6C3F1
Age/weight at study initiation: 10-14 weeks, male - 20.5-28.0 g; female 16.2-23.8 g
Source: Centre d'Elevage R., Janvier, France
Housing: Individually in Macrolon cages
Diet: Maintenance diet 9433LL Eberle Nafag AG, Gossau, Switzerland, *ad libitum*
Water: Tap water, *ad libitum*
Environmental conditions: 20-24°C
Temperature: 30-70%
Humidity: 12 hour light/dark
Photoperiod:
Acclimation period: 8-15 days

B. STUDY DESIGN:**1. In-life Study dates:**

Definitive Study	Start: January 9, 2002	End: February 8, 2002
Positive Control Study (Microscopy Study)	Start: April 20, 2000	End: May 19, 2000
Positive Control Studies (β -oxidation/S-phase)	Start: July 21, 2000	End: August 23, 2000

2. Animal assignment: Mice for the three studies were randomly assigned to the groups shown in Table 1.

Table 1. Study Design						
Test Material	Study Length (Days)	Group	Conc. (ppm)	Dose (mg/kg/day)	No. Males	No. Females
Blazer Technical (MRID 45693401)	28	0	0	0/0 ^a	5	5
	28	1	350	92/139	5	5
	28	2	1735	484/644	5	5
	28	3	5210	1346/1939	5	5
DINP (Microscopy Study; MRID 45686501)	28	0	0	0/0	5	5
	28	1	500	117/168	5	5
	28	2	1500	350/546	5	5
	28	3	4000	913/1272	5	5
	28	4	8000	1860/2807	5	5
DINP (β -oxidation/S-phase studies; MRID 45686502)	28	00	0	0/0	8	8
	28	10	500	115/142	8	8
	28	20	1500	352/441	8	8
	28	30	4000	951/1192	8	8

	28	40	8000	2022/2509	8	8
	7	00	0	0/0	8	8
	7	11	500	96/129	8	8
	7	21	1500	309/414	8	8
	7	31	4000	755/1121	8	8
	7	41	8000	1775/2511	8	8

^a Male/female**3. Dose selection rationale:**

Definitive Study: Doses were selected based on a previous study with mice that received 160, 800, and 2400 ppm (based on active ingredient). The duration of the study was not reported. The doses for the definitive study were corrected for test material active ingredient.

Positive Control Study: To correlate peroxisome proliferation with data obtained in a previous study, concentrations of 500, 1500, 4000, and 8000 ppm were used.

4. Diet preparation and analysis:

Definitive Study: For each diet in the test groups, an appropriate solution of the test material in acetone was prepared. The solution was sprayed on ~3 kg of diet under partial vacuum, heated to 40°C to remove the acetone, and adjusted to the proper concentration. The diets were prepared before the start of the study, frozen, and aliquots defrosted weekly for the four-week study. Stability of the test material was demonstrated in a previous study.

Homogeneity of the test material in the diet was determined in samples taken at the start and end of the study.

Positive Control Study: The test material was weighed and thoroughly mixed with a small amount of diet. The premix was diluted to the appropriate concentrations and kept frozen until use.

Results:**Homogeneity analysis:**

Definitive Study: The test material was shown to be within 7% of nominal concentration in three samples of the low- and high-concentration diets.

Positive Control Study: The positive control concentration was shown to be within 10% of nominal concentration in all diet preparations.

Stability analysis:

Definitive Study: The stability of the test material in the diet was shown to be >32 days in a previous study.

Positive Control Study: Over a period of 11 days, the positive control was proven to be within 97% of the nominal concentration when the diet was stored at room temperature.

Concentration analysis:

Definitive Study: The test material was shown to be within 7% of nominal concentration in samples of the low- and high-concentration diets.

Positive Control Study: The positive control concentration was within 10% of nominal concentration in all diet preparations.

5. **Statistics:** For the test material and DINP positive control (microscopy) studies, the mean and standard deviation for each parameter were calculated. Statistical differences were determined by Dunnett's test with $p \leq 0.05$. Statistical differences were determined using the Kruskal-Wallis test. Significant differences from control were determined by the Mann-Whitney U-test (two-sided). The level of statistical significance was $p \leq 0.05$.

C. METHODS:

1. **Observations:** For both studies, the mice were observed twice daily Monday through Friday and once on weekends and holidays for clinical signs of toxicity.
2. **Body weight:** Body weights for both studies were recorded prior to the start, on day 0 and weekly thereafter.
3. **Food consumption:** For the definitive study, food consumption was determined daily and food efficiency was calculated based upon individual values for body weight and food consumption.

For the positive control study, food consumption was determined weekly over 7 days and calculated as mean food consumption in grams/animal/day.

Food efficiency for the definitive study was calculated as the interval change in body weight divided by amount of food consumed times 100. Food efficiency for the positive control study (microscopy) was calculated by the reviewer using the above formula.

4. **Necropsy:** For the definitive and positive control (microscopy) studies, the test animals were sacrificed under Narcoren® anesthesia by perfusion fixation. Cacodylate buffer served as the rinsing solution and a 1% glutaldehyde solution in cacodylate buffer served as fixative. The

livers of all animals were removed, weighed, and sections from the lobus dexter medialis prepared for light and electron microscopy.

For the β -oxidation/S-phase positive control studies, the mice were killed by decapitation under CO₂ anesthesia. The liver, kidney, and brain were removed and weighed. (Other tissues were also collected and stored in 4% formalin, but the report does not state whether they were examined microscopically.) Two 5 mm strips of the right lateral and medial liver lobes of male and female mice treated for 1 or 4 weeks with DINP were fixed in 4% formalin for 24-48 hours and then stored in 70% ethanol. A section of the jejunum was also collected to serve as the positive control for the S-phase studies. Portions of the liver of male and female mice treated for 4 weeks with DINP were homogenized (buffering system not reported) for β -oxidation analysis (MRIDs 45686502 and 45693402).

5. **Measurement of Peroxisomes:** For male and female mice treated for 4 weeks with the test material or DINP, 10 cytoplasmic regions of hepatocytes were selected that fulfilled the following criteria:
- 1) the hepatocytes had to be adjacent to the central vein or in fields close by;
 - 2) no periportal hepatocytes were selected; and
 - 3) cytoplasmic fields that showed the highest number of peroxisomes were examined by electron microscopy. Sections examined under light microscopy were stained with diaminobenzidine (DAB). The ultrathin sections were examined at magnifications of 3150 and 6300 x. SIS imaging software system was used for an interactive peroxisome count and area measurement. From all measurements, the total number and area of peroxisomes was determined. The area occupied by peroxisomes was expressed in relation to the cytoplasmic region as percent. The peroxisomes were classified according to the following scheme:

Size Class (Grade)	μm^2
1	<0.1
2	<0.3
3	<0.5
4	<0.75
5	>0.75

6. **Palmitoyl-CoA Oxidation:** Peroxisomal β -oxidation of male and female mice treated with DINP for 4 weeks was determined by the measuring the cyanide insensitive oxidation of palmitoyl-CoA at 334 nm in liver homogenates according to the method of Lazarow (Enzym. 72; pp 315-319 (1981)). Palmitoyl oxidation was expressed as mU/mg protein or $\mu\text{M}/\text{min}/\text{mg}$ protein. Homogenate protein was determined by the biuret method
7. **S-phase Response:** Seven days before scheduled sacrifice, an osmotic minipump containing ~200 μL of 20 mg/mL bromodeoxyuridine (BrdU) was subcutaneously implanted in the dorsal region of all mice in the β -oxidation/S-phase positive control study. Following

sacrifice, a section of the right lateral and medial liver lobes and jejunum of males and females treated with DINP were processed for H&E (microscopic evaluation), immunochemical (BrdU incorporation), and TUNEL (apoptosis) staining. To prepare the slides to determine BrdU incorporation, the slides were deparaffinized, the DNA denatured with HCl, and the slides serially coated with mouse anti-BrdU, rabbit anti-mouse, streptavidin label, and Fast Red. The slides were then counterstained with hematoxylin.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed in any of the studies.
2. **Mortality:** None of the animals in the definitive study died. One 4000 ppm female in the positive control study (microscopy) died on day 16, however the death was considered unrelated to treatment. The deaths were considered unrelated to treatment.

In the β -oxidation/S-phase positive control studies, one male receiving 1500 ppm DINP and two males receiving 8000 ppm DINP for four weeks died. The deaths were considered unrelated to treatment.

- B. **BODY WEIGHT AND WEIGHT GAIN:** As shown in Table 2, the body weight of high-dose male mice in the definitive study was significantly decreased ($p < 0.01$) as much as 13% throughout the study. Likewise, the body weight gain of high-dose male mice was also decreased significantly ($p < 0.01$). However, the body weight of definitive study female mice was unaffected by treatment. In the positive control study (microscopy), the body weight of high-dose male mice was statistically significantly decreased 8% ($p < 0.05$) on study day 21. The body weight change of 4000 ppm male mice was significantly decreased during days 15 - 28 ($p < 0.05$) and for 8000 ppm males during days 7 - 28 ($p < 0.01$). No treatment-related effects were found with female mice. No biologically significant treatment-related effects on body weight or body weight change were found from male and female mice in the β -oxidation/S-phase studies (data not shown).

C. FOOD EFFICIENCY:

In the definitive study, food efficiency was decreased for high-dose male mice throughout the study and on day 14 for female mice (Table 3). No other treatment-related effects on food efficiency were identified. For the positive control study (microscopy), food efficiency was decreased in high-dose males during the first 21 days of the study, but was not decreased by study end. No treatment-related effects were found with female mice. No biologically

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significant effects on food consumption were found from male and female mice in the β -oxidation/S-phase positive control study (data not shown).

D. NECROPSY: No significant treatment-related effects were found in mice treated with Blazer Technical or DINP.

TABLE 2. Body weight (g) and body weight change (g) of mice fed diets containing Blazer Technical or DINP											
Test Material	Diet conc. (ppm)	Study day (males)					Study day (females)				
		0	7	14	21	28	0	7	14	21	28
Body Weight											
Blazer Technical ^a	0	27.2	28.0	28.7	29.4	30.3	23.0	24.2	25.0	25.0	25.4
	350	27.6	28.6	30.0*	30.4	31.2	22.4	23.5	23.9	24.3	25.2
	1735	27.3	28.6	29.9*	30.1	30.8	22.5	23.8	24.6	25.5	25.2
	5210	27.2	26.1**	25.6**	25.5***	26.7**	22.6	24.4	23.7	24.3	24.3
	Body Weight Change										
	0	—	0.8	1.5	2.1	3.1	—	1.2	2.0	2.1	2.5
	350	—	1.0	2.4	2.8	3.7	—	1.1	1.5	2.0	2.9
	1735	—	1.3	2.6	2.8	3.5	—	1.2	2.0	3.0	2.7
	5210	—	-1.1**	-1.6**	-1.7***	-0.5**	—	1.8	1.1	1.7	1.7
Body Weight											
DINP ^b	0	26.5	26.8	26.8	27.1	27.8	22.0	22.8	23.9	24.0	23.8
	500	27.2	26.8	26.9	27.3	27.8	22.2	22.9	23.1	23.2	23.3
	1500	27.1	27.5	26.9	27.4	27.6	21.9	22.4	23.2	23.0	23.0
	4000	27.2	27.2	26.4	26.3	27.2	21.9	23.5	23.4	23.4	23.9
	8000	27.1	26.3	26.1	25.0*	26.9	21.8	22.6	22.8	23.3	23.4
	Body Weight Change										
	0	—	0.2	0.3	0.5	1.3	—	0.9	1.9	2.1	1.8
	500	—	-0.4	-0.3	0.1	0.6	—	0.6	0.8	0.9	1.1
	1500	—	0.4	-0.2	0.3	0.5	—	0.5	1.3	1.1	1.1
	4000	—	-0.1	-0.9*	-0.9*	0.0*	—	1.6	1.5	1.5	2.0
8000	—	-0.8**	-1.0**	-2.2**	-0.2**	—	0.9	1.1	1.5	1.7	

^aData from pages 42-45, MRID 45693401

^bData from pages 44-47 of MRID 45686501

^cResults corrected for assumed error in Table IIA, page 64 of MRID 45693401; presumed body weight of mouse 20 is 25.6 g rather than 35.6 g.

*p \leq 0.05; **p \leq 0.01

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TABLE 3. Food efficiency (%) of mice fed diets containing Blazer Technical or DINP									
Test Material	Diet conc. (ppm)	Study Days (Male)				Study Days (Female)			
		7	14	21	28	7	14	21	28
Blazer Technical ^a	0	1.4	0.9	1.2	1.3	1.9	1.4	0.0	0.5
	350	1.8	2.8	0.7	1.4	1.9	0.5	0.7	1.4
	1735	2.0	2.3	0.2	1.3	1.6	1.3	1.4	-0.5
	5210	-3.2**	-1.2	-0.2 ^c	-1.9	3.1	-1.6**	1.0	0.2
DINP ^b	0	0.6	0.0	0.6	1.5	1.4	1.9	1.8	-0.3
	500	-0.9	0.2	0.9	1.2	1.4	0.3	0.2	1.2
	1500	0.9	-1.3	1.1	0.4	0.9	1.3	-0.3	0.0
	4000	0.0	-1.9	1.2	2.0	3.0	-0.2	0.0	1.1
	8000	-2.0	-0.4	-2.7	4.2	1.6	0.3	0.8	0.2

^aData from pages 46-47, MRID 45693401^bCalculated from Table 2 above and from Table 1A of MRID 45686501^cResults corrected for assumed error in Table IIA, page 64 of MRID 45693401; presumed body weight of mouse 20 is 25.6 g rather than 35.6 g

*p≤0.05; **p≤0.01

E. ORGAN WEIGHTS (β -oxidation/S-phase positive control studies): Other than a slight decrease in body weight (6%, p<0.01) and relative brain weight (18%, p<0.01) for high-dose females that received 8000 ppm test material for 28 days, treatment with DINP had no biologically significant effect on the terminal body weight or absolute or relative brain weights of male and female mice (Table 4).

However, treatment with DINP for 7 or 28 days significantly increased both absolute and relative liver weights in male and female mice fed 4000 ppm (16-20%) or 8000 ppm (40-45%). A slight increase in absolute liver weight was also found in male mice treated with 1500 ppm DINP (12%, p<0.05) for 28 days, however the relative liver weight of male mice in this group was not significantly increased. The relative liver weights of females were slightly increased following 7 days treatment (8%, p<0.01) or 28 days treatment (3%, p<0.05).

The absolute and relative kidney weights of male mice fed ≥4000 ppm and the absolute kidney weight of female mice fed 8000 ppm were statistically significantly decreased (9%, p<0.01) following 28 days of treatment with DINP.

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Table 4. Terminal body weight (g) and absolute (g) and relative to body (%) organ weights of mice fed DINP for 1 or 4 weeks.									
Diet Conc. (ppm)	Treatment Duration (days)	Males				Females			
		Body	Liver	Kidney	Brain	Body	Liver	Kidney	Brain
0 (absolute)	7	25.85	1.269	0.429	0.474	24.11	1.219	0.358	0.476
(relative)			4.909	1.653	1.833		5.052	1.480	1.975
500 (absolute)	7	26.24	1.246	0.411	0.479	23.61	1.190	0.341	0.485
(relative)			4.756	1.568	1.821		5.038	1.450	2.054
1500 (absolute)	7	26.48	1.330	0.420	0.481	23.93	1.301	0.351	0.488
(relative)			5.031	1.593	1.819		5.435**	1.470	2.043
4000 (absolute)	7	26.35	1.496**	0.405	0.476	23.68	1.44**	0.335	0.484
(relative)			5.673**	1.541	1.801		6.081**	1.414	2.047
8000 (absolute)	7	26.01	1.789**	0.398	0.481	23.89	1.768**	0.338	0.493
(relative)			6.881**	1.529	1.848		7.393**	1.413	2.066
0 (absolute)	28	25.85	1.269	0.429	0.474	24.11	1.219	0.358	0.476
(relative)			4.909	1.653	1.833		5.502	1.480	1.975
500 (absolute)	28	26.84	1.366	0.431	0.479	24.19	1.280	0.366	0.481
(relative)			5.091	1.601	1.785		5.292	1.505	1.994
1500 (absolute)	28	26.81	1.417*	0.424	0.479	24.10	1.289	0.371	0.491
(relative)			5.284	1.577	1.787		5.345*	1.537	2.048*
4000 (absolute)	28	26.15	1.558**	0.384*	0.484	23.79	1.524**	0.348	0.480
(relative)			5.937**	1.469**	1.858		6.411**	1.460	2.020
8000 (absolute)	28	26.03	1.853**	0.362**	0.478	22.73**	1.754**	0.324**	0.485
(relative)			7.095**	1.391**	1.842		7.723**	1.426	2.135**

Data from pp 68-76, MRID 45686502

*p≤0.05; **p≤0.01

F. LIVER STUDIES:**1. Light Microscopy:**

Definitive Study: In liver sections examined under light microscopy, only a few grade 1 peroxisomes (see size classification scheme above) were detectable in slides from control mice and male and female mice receiving 350 or 1735 ppm test material. In male and female mice receiving 5210 ppm test material, a slight to moderate number of grade 2 & 3 peroxisomes were found. In addition, an enhancement of the centrilobular region was also identified in these mice.

Positive Control Study (microscopy): In liver sections examined under light microscopy, only a few grade 1 or grade 2 peroxisomes (see classification scheme under Methods above) were detectable on slides from control mice and male and female mice receiving 500 or 1500 ppm DINP. No centrilobular enhancement was found. In male and female mice receiving 4000 or 8000 ppm DINP, a moderate number of grade 3 and grade 4 peroxisomes were found and enhancement of the centrilobular zone was clearly visible.

Positive Control Study (β -oxidation/S-phase): Following one week of treatment with 1500 ppm DINP, hepatocellular centrilobular hypertrophy was observed in 5/8 male and 1/8 female mice and in all male and female mice treated with ≥ 4000 ppm. After 4 weeks of treatment with DINP, centrilobular hypertrophy was found in 1/8 males treated with 500 ppm, 5/8 males and 2/8 females treated with 1500 ppm, and in all male and female mice treated with ≥ 4000 ppm that survived the study period.

In all groups treated for 7 days with DINP, increased mitotic figures were observed in zone 2 hepatocytes and were likely treatment-related (Table 5). However after 4 weeks of treatment, there was only a slight increase in the number of mitotic figures in males treated with 500 and 4000 ppm test material and the increase is of questionable biological significance.

Table 5. Liver mitotic figures in male and female mice treated for 7 or 28 days with DINP			
Diet Conc. (ppm)	Treatment Duration (days)	Zone 2 counts	
		Male	Female
0	7	2	8
500	7	41	16
1500	7	99	29
4000	7	40	45
8000	7	75	35
0	28	2	8
500	28	19	2
1500	28	6 ^a	4
4000	28	23	7
8000	28	4 ^b	10

Data from pages 10 and 11, MRID 45693402

^aN=7

^bN=6

Zone 1 = the portal zone; Zone 3 = the zone of the central vein; Zone 2 = the intermediate zone in between

- S-Phase Response (β -oxidation/S-phase):** Table 6 shows the S-phase response in positive control male and female mice following one or 4-weeks of treatment with DINP. Following one week of treatment with DINP, male mice treated with ≥ 500 ppm DINP had a significant dose-related increase (2-7 fold of control) in cell proliferation of all hepatocellular zones. After four weeks of treatment, the increase was still observable but was 1.5 to 2.1-fold of control and unrelated to dose.

Female mice treated with ≥ 1500 ppm DINP for one week also had a dose related 1.7-2.4-fold increase of all zones of hepatocytes. However after four weeks of treatment, only the 1500 and 4000 ppm groups still maintained a slight increase (1.4-1.6 fold of control) of cell proliferation relative to control mice.

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Table 6. Liver S-phase labeling index of male and female mice fed DINP for 7 or 28 days.									
Diet Conc. (ppm)	Treatment Duration (days)	Zone 1		Zone 2		Zone 3		All Zones	
		LI ^a	%	LI	%	LI	%	LI	%
Males									
0	7	0.11	100	1.04	100	0.08	100	0.41	100
500	7	0.19	173	2.18**	210	0.13	163	0.84*	205
1500	7	0.35**	318	2.76**	265	0.27**	338	1.14**	278
4000	7	0.47**	427	2.83**	272	0.75**	938	1.36**	332
8000	7	1.33**	1209	5.64**	542	1.60**	2000	2.90**	707
500	28	0.21	191	2.22*	213	0.16*	200	0.87*	212
1500	28	0.31**	282	1.29	124	0.24**	300	0.62	151
4000	28	0.28	255	1.26	121	0.26*	325	0.60	146
8000	28	1.11**	1009	1.19	114	0.14	175	0.83	202
Females									
0	7	1.27	100	3.48	100	0.64	100	1.79	100
500	7	1.17	92	3.20	92	0.78	122	1.71	96
1500	7	2.37	187	5.18	149	1.52*	238	3.04	170
4000	7	2.99**	235	5.97*	172	2.12**	331	3.69**	206
8000	7	3.60**	283	7.55**	217	2.14*	334	4.38**	245
500	28	0.92	72	2.48	71	0.59	92	1.32	74
1500	28	2.20	173	4.56	131	1.60*	250	2.80	156
4000	28	2.41	190	3.95	114	1.33	208	2.55	142
8000	28	1.60	126	1.71	49	0.51	80	1.27	71

Data from page 12, MRID 45693402

^aLI = Labeling Index

*p<0.05; **p<0.01

3. Apoptosis:

After treatment for 1 or 4 weeks with DINP, only a low number of apoptotic cells were identified in treated mice. The greatest number of apoptotic cells were found following one week of treatment in 4000 ppm and 8000 ppm male mice (1.8 - 2.8-fold increase in zone 2 hepatocytes) and in 1500 ppm female mice (~2.4-fold increase in all hepatocellular zones).

4. Electron Microscopy:

Definitive Study: Examination by electron microscopy showed a dose-related increase in size 1, 2, 3, 4 and 5 peroxisomes (see size classification above) in liver tissue of male and female mice treated with test material (Table 7) for 4 weeks. At 350 ppm, only slight increase of size 1 peroxisome (up to 1.5 fold; p<0.05) in males and females were observed.

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At 1735 ppm, significant increase in number of peroxisomes (2-6 fold; $p < 0.01$) in both sexes were observed. In both sexes of mice, dose-related significant increase ($p < 0.01$) in total peroxisomal area was apparent (2.7 fold increases in males and 2.2 fold increases in females).

At 5210 ppm, significant increase in number of peroxisomes in males (3-23 fold; $p < 0.01$) and females (1.5-35 fold; $p < 0.01$) were observed. In both sexes of mice, dose-related significant increase ($p < 0.01$) in total peroxisomal area was apparent (7.2 fold increases in males and 8.3 fold increases in females).

Positive Control Study (microscopy) (MRID 45686501): Examination by electron microscopy showed that DINP treatment for 4 weeks significantly increased the size, number, and cytoplasmic area of peroxisomes in male mice treated with ≥ 500 ppm and female mice treated with ≥ 1500 ppm (Table 7). Particularly increased were the number of microperoxisomes in mice treated with ≤ 4000 ppm DINP. There were also a dose-related increase in cytoplasmic volume of male and female mice treated with ≥ 1500 ppm positive control (ranging 1.8-9.7 fold increases in males and 1.5-6.0 fold increases in females).

TABLE 7. Quantitative evaluation of peroxisomes and cytoplasmic volume (%) in male and female mice fed Blazer Technical or DINP for 4 weeks.													
Test Material	Diet conc. (ppm)	Size Classification (males)						Size Classification (females)					
		1	2	3	4	5	Total Area of peroxisomes (in %)	1	2	3	4	5	Total Area of peroxisomes (in %)
Blazer Technical ^a	0	7.8	8.0	0.5	0.0	0.0	1.25	9.5	8.2	0.4	0.0	0.0	1.27
	350	11.8*	9.6	0.3	0.1	0.0	1.49	13.2*	7.5	0.21	0.0	0.0	1.25
	1735	15.4**	18.7**	2.9**	0.2*	0.0	3.43**	12.1	15.9**	2.2**	0.2	0.0	2.81**
	5210	22.3**	35.7**	11.4**	2.8**	0.3**	9.03**	14.6	35.7**	14.0**	4.3**	1.0*	10.6**
DINP ^b	0	12.1	10.7	1.0	0.1	0.1	1.93	24.6	12.1	1.4	0.1	0.0	2.48
	500	18.2*	11.0	1.3	0.0	0.0	2.16	22.3	9.3	0.5	0.0	0.0	1.88
	1500	55.0**	15.5	0.8	0.1	0.0	3.56**	32.4	19.9**	1.2	0.0	0.0	3.63*
	4000	49.6**	47.3**	10.9**	1.1**	0.1	10.6**	31.3	37.2**	11.1**	2.1**	0.1	9.52**
	8000	18.3**	35.5**	27.9**	13.9**	2.1**	18.7**	26.4	35.5**	18.6**	9.7**	1.8	14.8**

^aData from pages 28-29 MRID 45693401

^bData from page 29 of MRID 45686501

* $p \leq 0.05$; ** $p \leq 0.01$

5. **Peroxisomal β -oxidation:** After four weeks of DINP treatment, total hepatic peroxisomal β -oxidation was clearly increased relative to dose at all dietary concentrations in male mice (ranging 1.5-10.8 fold increase) and at concentrations ≥ 1500 ppm in female mice (ranging 1.7-8.3 fold increase) (Table 8).

Table 8. Peroxisomal β -oxidation (mU/mg) in male and female mice treated for 4 weeks with DINP				
Diet Conc. (ppm)	Males		Females	
	Mean	SD	Mean	SD
0	4.03	0.91	5.28	0.41
500	6.05**	1.42	5.91	0.82
1500	9.31***	2.00	8.88***	0.74
4000	23.1***	5.33	22.1***	1.60
8000	43.37***	2.18	43.9***	1.52

Data from page 32 of MRID 45686502

** $p \leq 0.02$; *** $p \leq 0.002$

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

Definitive Study: Based on the results, a slight to moderate increase in the number of peroxisomes within the centrilobular region of the high-dose group of both sexes treated with the test material was observed by light microscopy. No changes were found in male and female mice of lower dose groups. By electron microscopy, a dose-dependent increase in hepatic total cytoplasmic area in male and female mice treated with ≥ 1735 ppm was found. The author concluded that the development of different size classes of peroxisomes was related to treatment and that low doses increase the number of smaller peroxisomes and higher doses lead to an increase in number and size.

Positive Control Study (microscopic study): Dose-dependent increases of the number and size of hepatic peroxisomes in both sexes were found in male and female mice treated with ≥ 1500 ppm DINP. Male mice that received 500 ppm had an increase in the number of microperoxisomes that was considered related to treatment, although there was no significant increase in cytoplasmic area. No treatment-related effects were found in female mice that received 500 ppm test material.

Positive Control Study (β -oxidation/S-Phase): Dose-dependent increases of cyanide-insensitive palmitoyl-CoA oxidation were found in male mice treated with ≥ 500 ppm DINP and female mice treated with ≥ 1500 ppm. The increases of palmitoyl-CoA oxidation are consistent with the dose-related increase of peroxisome proliferation in treated mice. Significant increases of liver weight in male and female mice were found following one week of treatment with 1500 ppm (females only), 4000 ppm, and 8000 ppm DINP and after 4 weeks of treatment with ≥ 1500 ppm. The dose-dependent increases are consistent with

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centrilobular hypertrophy and peroxisome proliferation. The dose-related decreases in kidney weight were also considered to be treatment-related.

After one week of treatment with DINP, the S-phase response was greater than controls in all treatment groups. The increase of cell proliferation and the presence of mitotic figures were correlated and due to treatment. After four weeks of DINP treatment, only male mice in the 1500 and 4000 ppm groups showed more mitotic figures than control mice. In the remaining male and all female groups, mitotic figures were within the range of control mice.

Signs of apoptotic cell death were rare in control and DINP-treated groups. The data suggest, however, that the slight increase of apoptotic cells in 4000 ppm and 8000 ppm male mice after one week of treatment and the slight increase found in female mice treated with 1500 ppm for one week express a reactive and down-regulating mechanism and are an indirect effect of treatment-related cell proliferation

B. REVIEWER COMMENTS: The reviewer concurs with the study author's conclusion that treatment with Blazer Technical induced a dose-related increase in the number, size, and area of hepatic peroxisomes in mice treated with ≥ 350 ppm test material, thereby suggesting that **Blazer Technical is a weak peroxisome proliferator**. Also consistent with some types of peroxisome proliferation, there was a decrease in body weight and food efficiency of male mice receiving 5210 ppm test material. The reviewer also concurs with the study authors' interpretation of the positive control studies with diisononyl phthalate. Most alkyl-phthalates are known peroxisome proliferators and the results obtained from the positive control studies are consistent with what would be expected: dose-related increases in the four-enzyme cascade of palmitoyl-CoA oxidation, as well as dose-related increases in liver weight, peroxisomes, and centrilobular hypertrophy. Also consistent with treatment was the rapid increase of S-phase induction that was not sustained through the study. However, the reviewer considers the authors' suggestion that the DINP apoptosis results express a reactive and down-regulating mechanism and is an indirect effect of treatment-related cell proliferation to be questionable and inconsistent with the data. Typically, peroxisome proliferators do not affect apoptosis.

It should be noted that the two mechanism studies (S-phase response and enzyme induction) in the liver of mice treated with acifluorfen have been submitted by the registrant. These studies have been reviewed and the results of these studies also provide suggested evidence of peroxisome proliferation as a possible mechanism of action for liver tumors induction by acifluorfen. The following lists each study with the MRID number:

- 1) S-phase response study in the liver of mice following 3 days, 1 week, and 2 weeks feeding with acifluorfen (MRID45803601) and
- 2) Enzyme induction study in the liver of mice following 4 weeks feeding with acifluorfen (MRID 45793901).

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Peroxisome Proliferation - Mice (2002) Page 18 of 19
Nonguideline

C. **STUDY DEFICIENCIES:** None that would invalidate the studies.

DATA FOR ENTRY INTO ISIS

Nonguideline - Mouse

PC code	MRID	Study	Species	Duration	Doses (ppm)	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
114402	45693401, 45686501, 45686502, 45693402	Peroxisome proliferation	mouse	1 - 4 weeks	Blazer = 350, 1735, or 5210 ppm DINP = 0, 500, 1500, 4000, or 8000 ppm	Not applicable	Not applicable	Liver	Peroxisome proliferation

C

ACIFLUORFEN, SODIUM

ENZYME INDUCTION

EPA Reviewer: Irving Mauer, Ph.D.
 Registration Action Branch 3, HED (7509C)
 EPA Secondary Reviewer: Nancy McCarroll
 Toxicology Branch, HED (7509C)

Nancy McCarroll for I. Mauer
 Date: 02/06/03
Nancy E. McCarroll
 Date: 02/06/03

TXR No.: 0051328

DATA EVALUATION RECORD

STUDY TYPE: Enzyme induction in mouse liver (No OPPT/OECD Numbers.)DP BARCODE: D286776SUBMISSION CODE: S625019P. C. CODE: 114402TOX. CHEM. NO.: 755DTEST MATERIAL (PURITY): Blazer technical (46.1% a.i.)

SYNONYMS: Acifluorfen - Na(sodium salt); carbofluorfen.
Chemically: Sodium-5-[(2-chloro-4-trifluoromethyl)phenoxy]-2-nitrobenzoate (ANSI)

CITATION: Mellert, W., Deckardt, K., Beimborn, D. and van Ravenzwaay, B. (2002). Blazer Technical: Enzyme Induction Study in the Liver of B6C3F1 Mice - Oral Administration in the Diet for 4 Weeks, performed at the BASF Aktiengesellschaft Experimental Toxicology and Ecology, Lufwigshafen/Rhine (GERMANY). BASF Registration Document No. 2002/1011353, (Project No.: 99CO287/98157), September 3, 2002. MRID 45793901. Unpublished.

SPONSOR: BASF Corporation Agricultural Products Group, Research Triangle Park (NC)EXECUTIVE SUMMARY:

In an enzyme induction study (MRID 45793901), Blazer technical (46.1% acifluorfen -Na a.i. in acetone) was administered to groups of 10 male and 10 female B6C3F1 mice at dietary concentrations of 0, 350, 1735 and 5210 ppm (160, 800 and 2400 ppm of active ingredient) for 4 weeks. Mean daily intakes of the a.i. were 36.9/54.6, 180.0/255.3 and 709.1/933.5 mg/kg bw, [males/females], respectively. Food consumption and body weights were determined weekly. Animals were examined once a day, and a comprehensive clinical examination carried out weekly. In the first 5 animals of each group, glutathione concentration (GSH) in the liver was determined; in the second 5 animals, the amount of cyanide-insensitive palmitoyl-CoA-oxidation (PALCoA) in total protein was measured.

At the high-dose, significant ($p \leq 0.01$) decreases in male body weights were recorded on days 14, 21 and 28; the greatest decrease in male body weights (9.2%) occurred on Day 28. Body weight gain for this group was also significantly ($p \leq 0.01$) reduced compared to control at the same time intervals. No clear adverse effects were found on female body weight. There was a significant ($p \leq 0.01$) and dose-

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ENZYME INDUCTION

dependent increase in PALCoA, which is involved in peroxisomal fatty acid metabolism, ranging from a 58 to 576% increase for males and a 3 to 707% increase for females at 350 to 5210 ppm, respectively. Increased PALCoA was accompanied by decreased GSH concentrations in high-dose males (9% ↓) and females (15% ↓); the response was dose related for both sexes but only reached statistical significance in the high-dose females. **Based on these considerations, it was concluded that Blazer technical, containing 46.1% NA acifluorfen as the a.i., showed clear evidence of the induction of the peroxisomal enzyme system.** Thus, the demonstration of peroxisomal enzyme system induction in the liver satisfies one of the necessary criteria to classify a nongenotoxic substance as a peroxisome proliferator.

This study is classified as acceptable (non-guideline). Although this study does not satisfy the requirement for any current FIFRA Test Guideline 84-2, the results may be used in a possible mode of action analysis of the test substance.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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ENZYME INDUCTION

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Blazer technical (46.1% sodium acifluorfen)
 Description: Yellow-brown liquid
 Lot/Batch No.: 01501I300
 Purity: 46.1 % a.i. (Analytically verified: 44.9% by BASF Agro Product Center, 11/30/2001)
 Stability of compound: Stability guaranteed for 2 years at room temperature
 CAS No.: 62476-59-9
 Vehicle/Solvent used: Acetone
 Other comments: Test diets were analyzed for homogeneity, stability and actual concentrations.

2. Control Materials:
 Negative: None
 Solvent/final concentration: Acetone
 Positive: Diisononyl-phthalate (DINP).
 [See MRIDs 45686502 and 45693402]

3. Test Organisms: Mice.
 Strain: B6C3F1 Age: 9-10 weeks
 Weight:
 Males: 27.6 - 28.0 g Females: 22.7 - 23.0 g
 Source: Charles River, Sulzfeld, (GERMANY)
 Condition: Only animals free from clinical signs of disease were used in this study.

4. Test Compound Concentrations Used:
 Concentrations in the diet: 0, 350, 1735, 5210 ppm.
 Concentration of a.i.: 0, 160, 800, 2400 ppm.
 Mean daily intakes:
 Males: 0, 36.9, 180.0, 709.1 mg/kg Females: 0, 54.6, 255.3, 933.5 mg/kg [Relative to the a.i. (46.1%)]

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ENZYME INDUCTION

B. TEST PERFORMANCE

Following an acclimatization period, the test substance was administered daily in the diet for 4 weeks. The animals were examined for signs of toxicity or mortality twice a day during weekdays, and once on weekends and holidays. Food consumption was determined weekly and calculated as "mean food consumption in grams per animal and per day." Water consumption was observed daily for any changes in volume. Body weights were determined before the administration of compound, on day 0 at the start of the administration period, and thereafter at (4) weekly intervals. The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption by the following equation:

$$\frac{FC_x \times C}{BW_x} = \text{Substance intake for day X}$$

Where BW_x = Body weight on day X (in g)
 FC_x = Mean daily food consumption on day X (in grams)
 C = Concentration in the diet on day X (mg/kg)

1. Statistics of Clinical Examinations:

Statistical analyses of mean and SDs of each test group were performed according to the following table:

PARAMETERS	STATISTICAL TEST	MARKERS IN THE TABLES	REFERENCES
Food consumption, b.w., b.w. changes	A comparison of each group with the control (acetone) using Dunnett's test (two-sided) for the hypothesis of equal means	* for $p = 0.050$ ** for $p = 0.010$	Dunnett (1955) ¹ Dunnett (1964) ²

¹Dunnett, C.W. A multiple comparison procedure for comparing several treatments with a control. JASA 50; 1096 - 1121 (1955)

²Dunnett, C.W. New tables for multiple comparisons with a control. Biometrics 20; 482-491(1964).

2. Palmitoyl-CoA-Oxidation (second five of each group).

Animals were killed by cervical dislocation and decapitation, and the livers removed for the determination of cyanide-insensitive palmitoyl-CoA-oxidation (PALCoA) in the hepatic homogenates, employing a Cobas Fara enzyme analyzer provided by Roche Mannheim (GERMANY). Protein concentration was measured with a Hitachi 917 automatic analyzer provided by the same industrial laboratory. The analyses were carried out in a randomized sequence, according to the following methods:

PARAMETER	UNIT	METHOD	REFERENCES
Cyanide-insensitive palmitoyl-CoA-oxidation (PALCoA)	mp/mg protein or $\mu\text{mol/min}$ mg protein	Kinetic μv -test, 334 nm, 37°C	Lazarow (1981) ³
Total protein (TPROT)	g/L	Biuret	Weiselbaum (1946) ⁴

3. Statistics of Clinical Pathology:

Statistical analyses of means and SDs of each group were performed according to the following tables:

PARAMETER	STATISTICAL TEST	MARKER IN TABLES	REFERENCES
Cyanide-insensitive palmitoyl-CoA-oxidation (PALCoA)	Non-parametric one-way analysis using Kruskal-Wallis test (two-sided). If the resulting p-value was ≤ 0.05 , a pairwise comparison of each group was performed using Wilcoxon's-test (two-sided) for equal medians	* for $p = 0.050$ ** for $p = 0.010$	Siegel (1956) ⁵

³Lazarow, P.B. Enzym. 72, 315-319, (1981).

⁴Weiselbaum, T.E. Amer. J. Clin. Path. 10; 40 (1946); BM working instructions.

⁵Siegel, S. Non-parametric statistics for the behavioral sciences. McGraw-Hill, N.Y., 1956.

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4. **Glutathione (first five animals of each group)**

Animals were killed by cervical dislocation and decapitation. The livers were removed for the determination of glutathione concentration (GSH).

a. **Parameters:**

GSH-concentration in the liver was measured photometrically (with a Perkin-Elmer, Lambda 15, wavelength: 412 nm) at room temperature according to Sedlak *et al.* (1968)⁶. Liver S9-fraction from Aroclor 1254 - treated rats (from *in vitro* mutagenicity tests) served as the positive control.

b. **Statistics of Bioanalysis:**

Statistical analyses of means and SDs were carried out as follows:

PARAMETER	TEST	MARKERS IN TABLES	REFERENCES
Glutathione (GSH)	Pairwise comparison of each dose group with the control group using the Wilcoxon-test (two-sided) for the hypothesis of equal medians.	* for $p = 0.05-0.009$ ** for $p \leq 0.01$	Siegel (1956) ⁵

C. **RESULTS AND ASSESSMENT OF FINDINGS**

1. **Analyses:**

Homogeneity, Concentration and Stability Analysis

Chemical analyses revealed that the test material was homogeneously distributed throughout the feed: actual concentrations were within an acceptable range (93.75 - 98.5%) of the target; and the test material was stable in the diet for 32 days at room temperature.

⁶Sedlak *et al.*, Anal. Biochem. 25; 192-205 (1968).

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2. Clinical Examinations

No animals died during the study, and no abnormal clinical signs were observed. Food consumption was significantly increased ($p \leq 0.05$) in males administered the high dose (5210 ppm) in the diet on day 14. [However, the investigators ascribe this to spilling of food rather than reflecting increased food consumption]. High-dose males also lost weight ($p \leq 0.01$) on days 14, 21 and 28; body weight was 9.2% below control on day 28. (MRID 45793901, pp 35-38; ATTACHMENT TABLE 1A5 to 1A8). This was assessed as treatment-related. Females administered 1735 ppm weighed less ($p \leq 0.05$) on day 28. [However, the investigators assessed this as being incidental, since it was an isolated occurrence, lacking clear dose-response, and close to the control value]. (MRID 45793901, pp 39-40. ATTACHMENT TABLES 1A9 and 1A10).

3. Intake of Test Substance

The mean daily intake of test substance (mg/kg bw/d) over the entire study is shown in the following summary table:

TEST GROUP	CONCENTRATION IN DIET (PPM)	MEAN DAILY INTAKE(mg/kg)	
		MALES	FEMALES
1	350	80.1	1184
2	1735	390.5	553.8
3	5210	1538.2	2025.0

As related to a.i. (content: 46.1%):

TEST GROUP	CONCENTRATION IN DIET (PPM)	MEAN DAILY INTAKE (mg/kg)	
		MALES	FEMALES
1	160	36.9	54.6
2	800	180.0	255.3
3	2400	709.1	933.5

4. Palmitoyl-CoA Oxidation (second five animals of each group)

After 4 weeks of treatment, marked increases in cyanide-insensitive PALCoA were found in the liver homogenates of mid- and high-dose animals of both sexes (MRID 45793901, pp 41 and 42: ATTACHMENT TABLES 1B1 and 1B2), as

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shown in the following summary table. Slight, but statistically significant ($p \leq 0.05$) higher activities for PALCoA were also found in livers of low-dose males, but not low-dose females.

TEST GROUP	CONCENTRATION IN DIET (PPM)	PALCoA (mU/mg PROTEIN)	
		MALES	FEMALES
0	0	5.24	5.65
1	350	8.29** (+58%)	5.81 (+3%)
2	1735	25.29**(+383%)	17.84** (+216%)
3	5210	35.49** (+576%)	45.27** (+707%)

** $p \leq 0.01$

5. **Glutathione Concentration (first five animals of each group).**

After 4 weeks of treatment, marked decreases in hepatic GSH concentration (-15%) was found in high-dose females (only) (MRID 45793901, p 43: ATTACHMENT TABLE 1C-1); this was considered by the investigators to be biologically relevant. Although not reaching the level of statistical significance, decrease in GSH concentration in high-dose-males (~ 9%) was also considered of biological relevance by the investigators. The relevance of this effect was further proposed by the general trend of decreasing GSH-concentrations with an increasing dose in all treatment groups, as shown in the following summary table:

TEST GROUP	CONCENTRATIONS IN DIET (PPM)	GSH (μ MOL/G LIVER)	
		MALES	FEMALES
0	0	9.61	9.27
1	350	9.48	8.98
2	1735	9.07	8.95
3	5210	8.76	7.88**

** $p \leq 0.01$

B. INVESTIGATORS DISCUSSION/CONCLUSIONS

According to the investigators, the dietary administration of Blazer technical (46.1% acifluorfen-sodium) to B6C3F1 mice at concentrations of 350, 1735 and 5210 ppm for 28

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days (160, 800 and 2400 ppm, respectively, of the a.i.) resulted in hepatic peroxisome proliferation accompanied by: marked increases in the activities of certain peroxisomal enzymes, particularly those involved in the beta-oxidation of fatty acids, (*e.g.*, cyanide-insensitive PALCoA). Peroxisome proliferators increase peroxisomal beta-oxidation and P450-4A subfamily activity which is hypothesized to result in oxidative stress (O'Brien *et al.*, 2001)⁷.

In the study, PALCoA was increased 5 - 7 fold in high dose groups (both males and females), and dose-dependently in mid- and low-dose groups, and accompanied by dose-dependent decrease in hepatic GSH concentration in all groups, but only reaching statistical significance ($p \leq 0.05$) in high-dose females.

Thus, these findings are considered indicative of an induction of the peroxisomal enzyme system in the liver of Blazer technical-treated mice.

III. REVIEWERS' DISCUSSION/CONCLUSIONS:

A. Our reviewers agree with the investigators' conclusions regarding the induction of peroxisome enzymes in livers of mice administered Blazer technical (46.1% acifluorfen sodium) up to a clinically toxic dose (9.2% decrease in male body weight, and other effects at 2400 ppm a.i.). Thus the findings satisfy one of the criteria that is necessary to demonstrate before a non-genotoxic compound can be classified as a peroxisome proliferator.⁸

B. STUDY DEFICIENCIES:

None.

⁷O'Brien *et al.* : Effects of peroxisome proliferation on glutathione-related enzymes in rats and hamsters. *Toxicol. Appl. Pharmacol.*, 171; 27-37 (2001).

⁸Fricke, R.F. (2001). Lactofen: Assessment of Mode of Action on Liver Carcinogenicity. HED Memorandum to Christine Olinger, dated February 15, 2001.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

BODY WEIGHT

	Body Weight g Day 0	Body Weight g Day 7	Body Weight g Day 14	Body Weight g Day 21	Body Weight g Day 28
Male, GROUP 0 0 PPM					
Mean	28.0	28.5	28.5	29.5	30.3
SD	0.9	0.6	0.8	0.7	0.9
N	10	10	10	10	10
%dev					
Male, GROUP 1 350 PPM					
Mean	27.8	28.0	28.6	29.8	30.6
SD	1.3	1.0	1.0	1.0	1.0
N	10	10	10	10	10
%dev	-0.7	-1.9	0.4	0.7	0.8
Male, GROUP 2 1735 PPM					
Mean	27.6	28.4	29.5	30.5	31.6*
SD	1.0	0.9	1.1	1.0	1.2
N	10	10	10	10	10
%dev	-1.3	-0.6	3.3	3.1	4.2
Male, GROUP 3 5210 PPM					
Mean	27.7	27.8	26.0**	26.8**	27.5**
SD	1.1	0.9	1.2	1.3	1.2
N	10	10	10	10	10
%dev	-0.9	-2.6	-8.9	-9.3	-9.2

Key: D = Dunnett's test, Two-sided. * - 0.050. ** - 0.010
 Experimental Unit = Animal

BODY WEIGHT

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	Body Weight g Day 0 D	Body Weight g Day 7 D	Body Weight g Day 14 D	Body Weight g Day 21 D	Body Weight g Day 28 D
Female, GROUP 0 0 PPM					
Mean	23.0	23.4	23.6	23.8	25.6
SD	0.8	1.0	1.3	1.1	1.5
N	10	10	10	10	10
%dev					
Female, GROUP 1 350 PPM					
Mean	22.7	22.7	23.0	23.5	24.5
SD	0.9	0.9	0.8	1.2	1.4
N	10	10	10	10	10
%dev	-1.3	-3.0	-2.8	-1.0	-4.3
Female, GROUP 2 1735 PPM					
Mean	22.9	23.9	24.0	24.6	25.7
SD	1.0	1.1	1.0	1.3	1.6
N	10	10	10	10	10
%dev	-0.1	2.1	1.5	3.4	0.4
Female, GROUP 3 5210 PPM					
Mean	23.0	23.7	23.8	23.7	24.4
SD	0.7	0.4	0.6	0.7	0.8
N	10	10	10	10	10
%dev	0.2	1.2	0.6	-0.3	-4.6

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
 Experimental Unit = Animal

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BODY WEIGHT CHANGE

Study: 99C0287/98157

Print Date: 01/20/2002
Print Time: 08:21
Table : IA
Page : 7

	BW change g Day 7 D	BW change g Day 14 D	BW change g Day 21 D	BW change g Day 28 D
Male, GROUP 0 0 PPM				
Mean	0.6	0.6	1.6	2.4
SD	0.6	1.1	1.0	0.9
N	10	10	10	10
%dev				
Male, GROUP 1 350 PPM				
Mean	0.2	0.9	2.0	2.8
SD	0.5	0.8	1.0	1.2
N	10	10	10	10
%dev	-58.6	55.4	25.3	18.2
Male, GROUP 2 1735 PPM				
Mean	0.8	1.9*	2.9*	4.0**
SD	0.4	0.7	0.8	0.7
N	10	10	10	10
%dev	36.2	233.9	81.6	69.5
Male, GROUP 3 5210 PPM				
Mean	0.1	-1.7**	-0.9**	-0.2**
SD	0.8	1.2	1.4	1.3
N	10	10	10	10
%dev	-84.5	-405.4	-158.2	-107.2

Key: D = Dunnett's test. Two-sided. * = 0.050. ** = 0.010
Experimental Unit = Animal

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Study: 99C0287/98157

BODY WEIGHT CHANGE

Print Date: 08-29-2002
 Print Time: 08:29:21
 Table : IA
 Page : 8

	BW change g Day 7 0	BW change g Day 14 0	BW change g Day 21 0	BW change g Day 28 0
Female. GROUP 0 0 PPM				
Mean	0.4	0.7	0.8	2.7
SD	0.7	1.0	1.1	1.3
N	10	10	10	10
%dev				
Female. GROUP 1 350 PPM				
Mean	0.0	0.3	0.9	1.9
SD	0.4	0.5	0.7	0.8
N	10	10	10	10
%dev	-97.6	-53.8	7.5	-29.7
Female. GROUP 2 1735 PPM				
Mean	0.9	1.0	1.7	2.8
SD	0.6	0.8	1.0	1.0
N	10	10	10	10
%dev	123.8	58.5	106.3	4.9
Female. GROUP 3 5210 PPM				
Mean	0.7	0.7	0.7	1.4*
SD	0.5	0.7	0.8	0.9
N	10	10	10	10
%dev	54.8	12.3	-15.0	-46.6

Key: D - Dunnett's test, Two-sided. * = 0.050. ** = 0.010
 Experimental Unit = Animal

SUBSTANCE INTAKE

Study: 99C0287/98157

Print Date: 01-2002
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Table : 1A
Page : 9

	Subst.intake mg/kg Bw Day 7	Subst.intake mg/kg Bw Day 14	Subst.intake mg/kg Bw Day 21	Subst.intake mg/kg Bw Day 28
Male, GROUP 1 350 PPM				
Mean	78.6	87.2	73.8	80.8
SD	20.8	18.1	27.3	21.7
N	10	10	10	10
Male, GROUP 2 1735 PPM				
Mean	413.2	415.5	341.1	392.3
SD	101.2	46.1	82.8	65.9
N	10	10	10	10
Male, GROUP 3 5210 PPM				
Mean	1357.4	1930.2	1440.4	1424.7
SD	425.4	585.2	539.1	483.4
N	10	10	10	10

Key: Experimental Unit = Animal

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SUBSTANCE INTAKE

Study: 99C0287/98157

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Print Time: 10:02:52
Table : IA
Page : 10

	Subst.intake mg/kg Bw Day 7	Subst.intake mg/kg Bw Day 14	Subst.intake mg/kg Bw Day 21	Subst.intake mg/kg Bw Day 28
Female, GROUP 1 350 PPM				
Mean	120.7	124.9	108.2	119.7
SD	38.0	39.5	25.7	39.1
N	10	10	10	10
Female, GROUP 2 1735 PPM				
Mean	516.5	590.5	529.4	578.9
SD	105.1	156.4	95.3	160.0
N	10	10	10	10
Female, GROUP 3 5210 PPM				
Mean	2044.5	1919.2	2116.0	2020.1
SD	643.8	457.2	596.3	621.2
N	10	10	10	10

Key: Experimental Unit - Animal

BASF - DATATOX-F1 R11

ENZYMES

Print Date: 02-11-2002
Print Time: 15:41:32
Table : 1B
Page : 1

Study: 99C0287/98157

PAL CoA
mU/mg P.
Day 28
k

Male, GROUP 0 0 PPM

Mean 5.24
SD 0.50
N 5
%dev

Male, GROUP 1 350 PPM

Mean 8.29**
SD 0.57
N 5
%dev 58.15

Male, GROUP 2 1735 PPM

Mean 25.29**
SD 2.93
N 5
%dev 382.78

Male, GROUP 3 5210 PPM

Mean 35.41**
SD 2.49
N 5
%dev 575.91

Key: k = Kruskal-Wallis + Wilcoxon-test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

BASF Aktiengesellschaft
Project No 99C0287/98157

Table IC.1

Summary Data

LIVER EXAMINATION (mouse)

Nominal days in study 28

GSH - Concentration

Males Group [ppm]					Females				
	0	1	2	3		0	1	2	3
	0	350	1735	5210		0	350	1735	5210
	GSH [μmol/g liver]	GSH [μmol/g liver]	GSH [μmol/g liver]	GSH [μmol/g liver]		GSH [μmol/g liver]	GSH [μmol/g liver]	GSH [μmol/g liver]	GSH [μmol/g liver]
Mean	9.81	9.48	9.07	8.76		9.27	8.98	8.95	7.88
SD	1.02	0.96	0.63	0.41		0.77	0.61	0.24	0.35
N	5	5	5	5		5	5	5	5

Statistics Wilcoxon-test (two-sided): *p<=0.05. **p<=0.01 (Statistical unit = animal)

D

ACIFLUORFEN, SODIUM

DNA SYNTHESIS (S-PHASE RESPONSE)/CELL PROLIFERATION

EPA Reviewer: Irving Mauer, Ph.D.

Registration Action Branch 3, HED (7509C)

Secondary Reviewer: Nancy McCarroll

Toxicology Branch, HED (7509C)

Date: 02/06/03

Date: 02/06/03

TXR No.: 0051328

DATA EVALUATION RECORD

STUDY TYPE: Hepatic DNA synthesis/cell proliferation in mice [No OPPTS/OECD numbers]DP BARCODE: D286776SUBMISSION NO. S625019PC CODE: 114402TOX. CHEM. NO.: 755DTEST MATERIAL (PURITY): Blazer Technical (46.1% a.i.)SYNONYMS: Acifluorfen-Na

CITATION: Mellert, W., Kaufmann, W., van Ravenzwaay, B. (2002). Blazer Technical: S-Phase Response Study in the Liver of B6C3F1 Mice - Administration in the Diet for 3 Days, 1 Week and 2 Weeks, conducted in BASF's Aktiengesellschaft Experimental Toxicology and Ecology Laboratory, Ludwigshafen/Rhine (GERMANY). BASF Registration Document No. 2002/1011947, Project No. 99CO287/98153, dated October 18, 2002. MRID 45803601. Unpublished.

SPONSOR: BASF Corporation Agricultural Products Group, Research Triangle Park, NCEXECUTIVE SUMMARY:

In a DNA synthesis (S-phase response)/cell proliferation study (MRID 45803601), Blazer Technical (46.1% sodium acifluorfen in acetone) was administered to groups of 8 male and 8 female B6C3F1 mice at dietary concentrations of 0, 350, 1735 and 5210 ppm (i.e.: 160, 800 and 2400 ppm of the active ingredient), mean daily intakes for males/females of 40/54, 227/287 and 714/845 mg/kg/day, respectively, for 3 days, 1 week or 2 weeks. Food consumption and body weights were determined weekly. The animals were examined at least once a day; and additionally, comprehensive clinical examination was performed weekly. One week prior to necropsy, osmotic mini-pumps containing bromodeoxyuridine (BrdU) were implanted subcutaneously. Cell proliferation (S-phase response) and apoptosis were determined in the liver.

No animals died during the study and no clinical signs were observed. The test material had no effect on food or water consumption, and there were no consistent effects on male or female body weight.

Dose-related increases in liver weights (generally both absolute and relative) and findings of moderate or

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DNA SYNTHESIS (S-PHASE RESPONSE)/CELL PROLIFERATION

moderate to severe increases in panlobular hypertrophy of hepatocytes were seen in males and females exposed to 714/845 a.i. mg/kg/day, respectively, on all treatment schedules (3-day, 1- and 2-week). Liver hypertrophy was most pronounced in the males and peaked after 1 week of treatment. By 2 weeks, an increase in single cell necrosis and apoptotic cells was observed primarily in high-dose males. This finding, suggesting cytotoxicity to the target organ, indicates that dosing was adequate to assess cell proliferation in the liver. **The oral administration of Blazer Technical (46.1% acifluorfen-sodium) to mice produced a dose-dependent and significant induction of BrdU labeling in the liver, which is indicative of cell proliferation (S-phase response), in all dose groups. The most pronounced effect for each of these three parameters (↑ liver weight, ↑ liver hypertrophy and ↑ BrdU labeling) for both sexes was seen after 1 week of treatment.**

This study is classified as acceptable (non-guideline). Although this study does not satisfy the requirement for any current FIFRA Test Guideline 84-2, the results may be used in a possible mode of action analysis of the test substance.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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DNA SYNTHESIS (S-PHASE RESPONSE)/CELL PROLIFERATION

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Blazer Technical
 Description: Yellow-brown liquid.
 Lot/Batch No.: 01501I300
 Purity: 46.1% acifluorfen - Na
 Stability: Guaranteed over the exposure period (Certification of Analysis 44.9% according to BASF, November 27, 2001).
 CAS No.: 62476-59-9
 Vehicle/Solvent: Acetone
 Other comments: a. Homogeneously distributed in feed according to analysis on February 18/19, 2002.
 b. Storage: Room temperature.
2. Control Materials:
 Negative: None
 Solvent: Acetone
 Positive (concentration, solvent): [No positive control substance was employed.]
3. Test Compound Concentrations:
 Preliminary Toxicity Test (Not performed.)¹
Main Assay:
 Dietary concentrations: 0, 350, 1735, 1510 ppm
 Concentration of a.i.: 0, 160, 800, 2400 ppm
4. Medium (Food): Basic maintenance diet for rodents, meal from Provimi KLIBA SA., Kaiseraugst (SWITZERLAND)
5. Test Animals:
 Species: mice Strain: B6C3F1 Age: 9 - 11wks. Weight: See tables.
 Supplier: Charles River, Sulzfeld (GERMANY)

NOTE: Only animals free of clinical signs of disease were used in this study.

6. Test Groups and Doses
 Groups were divided into two subsets: Subset A, first 4 males and 4 females;
 Subset B, the second 4 males and 4 females:

¹Doses were selected to approximated doses (160, 800 and 2400 ppm, a.i.) used in a "conducted long term study."

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Dose Group	Treatment Period	Concentration in the Diet (ppm) ²	No. of Animals Male	No. of Animals Female
00	1 wk	0	1 - 8	89 - 96
01	1 wk	350	9 - 16	97 - 104
02	1 wk	1735	17 - 24	105 - 112
03	1 wk	5210	25 - 32	113 - 120
11	3 days	350	33 - 40	121 - 128
12	3 days	1735	41 - 48	129 - 136
13	3 days	5210	49 - 56	137 - 144
20	2 wks	0	57 - 64	145 - 152
21	2 wks	350	65 - 72	153 - 160
22	2 wks	1735	73 - 80	161 - 168
23	2 wks	5210	81 - 88	169 - 176

²With respect to the purity of the test substance (46.1%), the concentrations of the active ingredient were 0, 160, 800 and 2400 ppm.

7. Osmotic Mini-pumps:

On scheduled dates, osmotic mini-pumps (Alzet Osmotic Pump, Type 2001, Alzet Corporation, Palo Alto, CA; supplied by SAVO, Kisslegg, GERMANY), filled with 200 µL of 5-bromo-2-deoxyuridine, BrdU in 20 mg/mL physiological saline, from Servat Feinbiochemikalien, Heidelberg, (GERMANY) were implanted subcutaneously in the back of respective animals 7 days prior to necropsy, under ISOFLOR® anesthesia (Essex, Munich, GERMANY). Dosing period of the pumps was 7 days.

B. TEST SUBSTANCE PREPARATION AND ANALYSIS

1. Preparation:

For each concentration of substance incorporated food, an appropriate solution of the test substance in acetone was prepared, and sprayed on about 3 kilograms of food under partial vacuum in a laboratory evaporator. Then, the acetone was removed by treating food at 40°C for 60 minutes. This premix was adjusted to the desired concentration with an appropriate amount of food, and mixed in a laboratory mixer for 10 minutes. The mixtures was prepared once before the start of the study.

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2. Analyses:

Analyses were carried out at the analytical Laboratory of the Experimental Toxicological and Ecology of BASF Aktiengesellschaft.

The stability of test substance was determined prior to the study. Homogeneity analyses of test substance preparations were determined in samples of the highest and lowest concentrations at the start of the administration period; these samples also served for concentration control analyses. Additional concentration control analyses were conducted with samples drawn from the mid-concentration at the start of the study. [The methods used for analytical investigations of test substance preparations are listed in Volume III (Supplement) of the submission (MRID No. 45803601)].

Food, water and bedding were regularly assayed for contaminants.

C. EXPERIMENTAL PROCEDURE AND TIME SCHEDULE

1. Following an acclimatization period, the animals were distributed randomly by weight, separately by sex. The weight variation of the animals did not exceed 20% of the mean weight of each sex.

The control diet and diets containing increasing concentrations of test substance were administered daily for 3 days, 1 or 4 weeks. The mini-pumps were implanted one week prior to necropsy (dosing period of pumps = 7 days). All surviving animals were sacrificed without fasting.

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DNA SYNTHESIS (S-PHASE RESPONSE)/CELL PROLIFERATION

The study was performed according to the following time table:

Section A (Male Animals)	Section A (Female Animals)	Section B (Male Animals)	Section B (Female Animals)	Dose Group	Phase of Study/ Examinations	Day of Study
2/ 12	2/12	2/12	2/12		Experimental starting date: arrival of the animals and start of the acclimatization period	
2/14	2/14	2/14	2/14	00 - 03	Randomization	- 4 - 11 - 6 - 13
2/18	2/25	2/20	2/27	00 - 03	Start of administration period and implantation of BrdU mini-pumps	0
2/18	2/25	2/20	2/27	11 - 13	Implantation of BrdU mini-pumps	0
2/22	3/1	2/24	3/3	11 - 13	Start of administration period	4
2/25	2/26	2/ 26	2/26	20 - 23	Start of administration period	0
3/4	3/5	3/4	3/5	20 - 23	Implantation of BrdU mini-pumps	7
2/25	3/4	2/27	3/6	0 - 03	Last weighing, necropsy	7
2/25	3/4	2/27	3/6	11 - 13		7
3/11	3/12	3/11	3/12	20 - 23		14
March - September 2002				Evaluation and Reporting		
October 01, 2002				Experimental completion date; draft report to QAU		

D. CLINICAL EXAMINATIONS

1. Clinical Observations:

The animals were examined for signs of toxicity or mortality twice-a-day on weekdays, and once-a-day on weekends and public holidays. Additional general clinical examinations were performed once-a-week.

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2. Food Consumption:

Food consumption was determined weekly, and calculated as mean food consumption in grams per animal and day.

3. Water Consumption:

Water consumption was observed daily by visual inspection of water bottles for any changes in volume.

4. Body Weight Data:

Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, body weight was determined on days 0 (start of administration period), 4, 7, and 14.

5. Intake of Test Substance:

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption, as follows:

$$\frac{F C_x \times C}{B W_x} = \text{Substance intake for Day } x$$

Where: BW_x = Body weight on Day x (in g)

FC_x = Mean daily food consumption on Day x (in g)

C = Concentration in the diet on Day x (mg/kg)

6. Statistics of Clinical Examinations:

Means and SDs of each test group were calculated for several parameters (see tables in report, MRID 45803601; further statistical analyses were conducted according to the following table:

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Parameters	Statistical Test	Markers in the Tables	References
Food consumption, body weight	A comparison of each group with the control group using Dunnett's test (two-sided) for the hypothesis of equal means	* for $p = 0.050$ ** for $p = 0.010$	Dunnett, C. W. (1955) ³ Dunnett, C.W. (1964) ⁴

* for $p \leq 0.05$.** for $p \leq 0.01$.

E. PATHOLOGY

1. Necropsy:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. The exsanguinated animals were necropsied, and assessed by gross pathology.

2. Weight Parameters:

The following weights were determined:

a. Anesthetized animals

b. Liver

3. Organ/Tissue Preservation List:

The following organs and tissues were preserved in neutral buffered 4% formaldehyde:

³Dunnett, C.W.: A multiple comparison procedure for composing several treatments with a control. JASA 50, 1096 - 1121 (1955)

⁴Dunnett, C.W.: New tables for multiple comparisons with a control. Biometrics, 20, 482-491 (1964)

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i	All gross lesions	ii	Brain
iii	Pituitary	iv	Thyroid/Parathyroid
v	Thymus	vi	Trachea
vii	Lungs	vii	Heart
ix	Liver	x	Gall bladder
xi	Spleen	xii	Kidneys
xiii	Adrenals	xiv	Testes
xv	Epididymides, prostate, seminal vesicles	xvi	Female mammary glands
xvii	Ovaries	xviii	Oviducts, uterus, vagina
xix	Stomach (fore- and glandular)	xx	Duodenum, jejunum, ileum
xxi	Cecum, colon, rectum	xxii	Urinary bladder
xxiii	Lymph nodes	xxiv	Sciatic nerve
xxv	Bone marrow (femur)	xxvi	Eyes
xxvii	Spinal cord (cervical, thoracic, lumbar)	xxviii	Skin

4. **Histotechnical Processing:**

The livers were sliced in two 5 mm-thicknesses (right lateral and medial lobes), fixed in 4% formaldehyde solution for 24-48 hours, and stored in 70% ethanol. (A section of jejunum was included as positive control.) After fixation, histochemical and immunohistological processing and examination by light microscopy were carried out according to the following table:

Organs	Test Groups (3-day, 1-week, and 2-week treatment)			
	Control	350 ppm	1735 ppm	5210 ppm
Liver	A8,I8	A8,I8	A8,I8	A8,I8
Jejunum	A8,I8	A8,I8	A8,I8	A8,I8

Methods and scope of examination:

A = Hematoxylin and Eosin stain
 I = Immunohistology
 8 = All animals per group

The section of jejunum was included to confirm the supply of BrdU to the tissues; however, the jejunum was not evaluated for determination of DNA synthesis or histopathology.

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From each block, 5 sections were cut at 3 μ m: one was used for hematoxylin-eosin (H&E) staining, and 4 others for immunohistology, two of these serving as test slides for the calculation of the S-phase (BrdU stain) and apoptosis (TUNEL stain), respectively. The other two served as negative controls for each stain. The tissues were mounted on slides covered with 1% 3-aminopropyl-triethoxysilene (APTS, supplied by Servat).

The H&E stained slides were examined, and assessed for an estimation of the amount of mitotic and apoptotic figures with respect to their morphological features. Slides were not coded prior to examination.

5. Staining Procedures - Immunohistology

BrdU Stain:

The slides were dewaxed, hydrated and covered with phosphate-buffered saline (PBS). The following staining procedure was performed on all slides at room temperature in an automated cell staining system (Optimax, from BioGenex, Hamburg, FRG, GERMANY). After DNA denaturation with hydrochloric acid, the slides were incubated consecutively with a primary antibody (mouse anti-BrdU), a secondary antibody (rabbit anti-mouse), a streptavidin enzyme label, and a chromagen substrate solution (Fast Red). Sections were then counterstained with Mayer's hematoxylin only (from BioGenex).

TUNEL:

TUNEL staining was performed according to the protocol of the TUNEL kit (from Roche Diagnostics, Mannheim, FRG).

Quantitative Measurements of S-Phase Response and Apoptosis.

Positively-stained cells are characterized by a red reaction product covering the nuclei (BrdU plus apoptosis). Light microscopy at 200 X using an image analysis system (Quantimed 500, from Leica; or KS 400, from Zeiss, GERMANY) provided discrimination of hepatocytes from mesenchymal cells on the basis of size and shape. Apoptotic cells - those showing a positive reaction - were differentiated from necrotic cells by morphology. Labeled and unlabeled cells were counted by true ("genuine") color detection.

Cell proliferation in the liver can be diffusely induced in all hepatocytes or localized in specific regions of the lobule. The liver lobule is, therefore, subdivided into 3 zones

according to Rappaport (1963,1980)^{5,6} comprising the portal zone (zone 1), the zone of the central vein (zone 3), and the intermediate zone (zone 2). According to Bahnemann and Mellert (1997)⁷ and Goldsworthy *et al.* (1991, 1993)^{8,9}, a total of more than 1000 cells per zone, *i.e.*, more than 1000 cells per animal, was assessed.

The labeling index (LI), in BrdU-immunostained slide was calculated as follows:

$$\text{BrdU LI (\%)} = \frac{\text{Labeled Cells}}{\text{Total No. of Labeled and Unlabeled Cells}} \times 100$$

Due to the small number of positively-labeled apoptotic cells in general, the total numbers of apoptotic cells were counted in whole liver sections present on slides. The total counts are shown in the attached tables (ATTACHMENT Tables). No labeling index (LI) was calculated.

6. **Statistics of Pathology**

Means and SDs of each test group were calculated for the variables of terminal body weight, and of absolute and relative organ weights (related to terminal b.w.) of animals in each test group. Further statistical analyses were performed according to the following table:

⁵Rappaport, A.M. Acinar units and the pathophysiology of the liver. *In*: The Liver, Vol 1 (ed.: Ch Rouillier). Academic Press, N.Y., 1963: pp 265-328.

⁶Rappaport, A.M.: Hepatic blood flow: Morphologic aspects and physiologic regulation. *In*: Liver and Biliary tract Physiology (ed. : N. Javitt). International Review of Physiology, vol. 21, University Press, Baltimore (MD), 1980.

⁷Bahnemann, R. and Mellert, W.: Lobule-dependent zonal measurement (LZM) method for the determination of cell proliferation. *Exp.. Toxicol. Pathol.* **49**: 189-196 (1997).

⁸Goldsworthy, T. L., Morgan, K.T., Popp, J. A. and Buttersworth, B. E.: Guidelines for measuring chemically induced cell proliferation in specific rodent target organs. *In*: Chemically-Induced Cell Proliferation. Implications for Risk Assessment, Wiley-Liss, Inc., N.Y. (1991), pp 253-284.

⁹Goldsworthy, T.L., Buttersworth, B. E. and Maronpot, R. R.: Concepts; labeling procedures and design of cell proliferation studies relating to carcinogenesis. *Environ. Hlth. Perspec.* **101**: 59-66 (1993).

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Parameters	Statistical Test	Markers in the Tables	References
Weight parameters, data of S-Phase Response and Apoptosis	A pairwise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.	* for $p \leq 0.05$ ** for $p \leq 0.01$	Nijenhuis, A. and S.W. Wilf (1978) ¹⁰ Hettmannsperger (1984) ¹¹

II RESULTS AND ASSESSMENT OF FINDINGS

[NOTE: When intergroup differences are referred to as “significant,” the investigators stated that this meant that the differences have attained statistical significance (at least $p \leq 0.05$), when compared with the control group.]

A. DIETARY ANALYSES:

1. **Stability Analysis:**

The stability of the test substance in the diet for 32 days at room temperature was verified (by analysis provided in Volume III of the submission, MRID 45803601).

2. **Homogeneity and Concentration of Control Analyses:**

The homogeneity/concentrations of test substance preparations were demonstrated. The mean value was in an acceptable range (98.4% - 107.6% of the target concentrations, as provided in Vol III of MRID 45803601).

B. CLINICAL EXAMINATIONS

1. **Mortality:** No animals died during the study.

2. **Clinical Observations:**

No substance-related findings were observed (MRID 458003601, pp 46-51 - ATTACHMENT Tables 1A-1 to 1A-6).

3. **Food Consumption:**

No substance related findings were obtained (MRID 45803601, pp 52-57 - ATTACHMENT Tables 1A-7 to 1A-12).

¹⁰Nijenhuis, A. and S.W. Wilf: Combinatorial Algorithms. Academic Press, N.Y. (1978), pp 32-33

¹¹Hettmannsperger, T.P.: Statistical Inference Based on Ranks. John Wiley and Sons, N.Y. (1984), pp 132-140.

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4. **Water Consumption:** No overt changes in volume consumed were observed.
5. **Body Weight Data:**
(Figures 1-6; MRID 45803601, pp 58-63 ATTACHMENT Tables 1A-13 to 1A-18).

Body weight was significantly decreased in high-dose males of Group 03 on Day 4 (-6%), of Group 13 on Day 7 (-8%), and of Group 23 on Day 14 (-8%). All of these changes were assessed by the study investigators as treatment-related. However, the findings occurred sporadically and were not seen uniformly in all 3 subsets of males treated with 5210 ppm (see Summary Table presented below.). The significantly increased value of mid-dose males (Group 22) on day 7 was considered incidental by the study authors. No effects were seen on female body weights.

6. **Intake of Test Substance:**
The mean daily test substance intake over the entire study period (MRID 45803601, pp 64-69; ATTACHMENT Tables 1A-19 to 1A-24) is presented in a summary table shown below (p. 14):

Test Group (Subsets)	Concentration in the Diet (ppm)	Mean Daily Test Substance Intake (mg/kg bw/d)	
		Males	Females
1	350	83.8	112.0
2	1735	499.6	600.4
3	5210	1652.4	1595.1
11	350	82.9	121.6
12	1735	527.0	641.9
13	5210	1498.6	1802.2
21	350	90.6	119.3
22	1735	451.0	625.2
23	5210	1493.7	2098.

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Related to the active ingredient (purity: 46.1%):

Test Group	Concentration in the Diet (ppm)	Mean Daily Substance Intake (mg/kg bw/d)	
		Males	Females
1	160	38.6	51.6
2	800	230.3	276.8
3	2400	761.8	735.3
11	160	38.2	56.1
12	800	242.9	295.9
13	2400	690.9	830.8
21	160	41.8	55.0
22	800	207.9	288.2
23	2400	688.6	967.5

The following mean substance intake of the active ingredient is given in the following summary table:

Concentration in Diet (ppm)	Mean Daily Test Substance Intake (mg/kg bw/d)	
	Males	Females
160	40	54
800	227	287
2400	714	845

C. PATHOLOGY:

1. **Weighted Parameters:**

When compared with the control groups, the following weight deviations were noted: (MRID 45803601: pp 70-81; ATTACHMENT Tables 1B-1 to 1B-12), summarized as follows:

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3 Day Treatment												
Test Group (ppm)	Terminal Body Weights				Absolute Liver Weights				Relative Liver Weights			
	Males (%)		Females (%)		Males (%)		Females (%)		Males (%)		Females (%)	
	+	-	+	-	+	-	+	-	+	-	+	-
350		2		1		4	1			1	2	
1735		2		0	14**		17**		16**		16**	
5210		12**		4	5		46**		20**		52**	

* Statistically significant (Kruskal-Wallis -H- plus WILCOXON test, two-sided, $p \leq 0.05$)

** Statistically significant (Kruskal-Wallis -H- plus WILCOXON test, two-sided, $p \leq 0.01$)

Significant gains in absolute and relative liver weights of females of the 1735- and 5210-dose groups were seen. In the high-dose groups, the gain is more pronounced in the females (*i.e.*, only 5% in absolute liver weight for the high-dose males.) The male top dose group exhibited a significant decrease in the terminal body weights.

1-week Treatment												
Test Group (PPM)	Terminal Body Weight				Absolute Liver Weights				Relative Liver Weights			
	Male (%)		Female (%)		Male (%)		Female (%)		Male (%)		Female (%)	
	+	-	+	-	+	-	+	-	+	-	+	-
350		2	1		5		4		7*		3	
1735		1	3		29**		40**		30**		36**	
5210		7**		1	40**		80**		50*		83**	

* Statistically significant (Kruskal - Wallis -H- plus WILCOXON Test, two-sided, $p \leq 0.05$)

** Statistically significant (Kruskal - Wallis -H- plus WILCOXON Test, two-sided, $p \leq 0.01$)

For mice sacrificed after the 1-week treatment, there is a significant and dose-dependent gain in liver weights (both absolute and relative) in the mid- and high-dose males and females. High-dose males exhibited a significant decrease in the terminal body weights.

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2-week Treatment												
Test Group	Terminal Body Weight				Absolute Liver Weights				Relative Liver Weights			
(ppm)	Males (%)		Females (%)		Males (%)		Females (%)		Males (%)		Females (%)	
	+	-	+	-	+	-	+	-	+	-	+	-
350	0		0		8**		1		8**		1	
1735	2			1	40**		24**		37**		25**	
5210		11**		3	25**		68**		40**		74**	

* Statistically significant (Kruskal - Wallis -H - plus WILCOXON Test, two-sided, $p < 0.05$)

**Statistically significant (Kruskal - Wallis -H - plus WILCOXON Test, two-sided, $p < 0.01$)

After 2 weeks of treatment, there was a significant and dose-dependent gain in male liver weights (absolute and relative) in all dose groups. The terminal body weight for males was also significantly reduced. For females, a significant and dose-dependent gain in absolute and relative liver weights was apparent for the mid- and high- dose groups.

2. Gross Lesions:

An "enlarged liver" was noted in all females (8/8) test animals of the top dose group after the 1-week treatment, and in 7 females (7/8) after the 2-week treatment, respectively. (MRID 45803601, pp 82-84: Report Tables 1B-13 to 1B-15).

All other gross lesions are considered to be incidental and not related to treatment.

3. Histopathological Findings:

Codes used at finding level:

The codes are used for a grading system which takes into consideration either the severity or the number or the size of a microscope finding is presented below:

Grade	Severity	Number	Size
1	Minimal	Very few	Very small
2	Slight	Few	Small
3	Moderate	Moderate number	Moderate size
4	Marked	Many	Large
5	Massive	Extensive number	Extensive size

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In the high-dose groups, a panlobular hypertrophy of hepatocytes was recorded after the 3-day, 1-week, and the 2-week treatment periods (MRID 45803601, pp 85-87: Report Tables 1B-16 to 1B-1). The gradings were moderate for the males and moderate or severe for the females. In the mid-dose males and females, centrilobular hypertrophy was noted after the 3-day, 1-week, and 2-week treatment periods; the gradings ranged between slight and moderate.

In the low-dose groups, a centrilobular hypertrophy was diagnosed after the 1-week treatment for 7 males (7/8), after the 3-day treatment for six males (6/8) and 1 female (1/8), and after the 2-week treatment for all males (8/8) and two females (2/8). The gradings ranged between minimal and slight.

“Single cell necrosis” was recorded only for the high-dose groups and was pronounced and accompanied by a mononuclear cell infiltration (inflammatory response) after 2-weeks of treatment in males. The gradings of the single cell necrosis ranged between minimal and moderate after the 2-week treatment period. After the 3-day and the 1-week treatment periods the finding of a single cell necrosis was only found in single cases and only up to a minimal extent. The diagnosis of single cell necrosis includes the observation of hyaline droplets and apoptotic cell bodies.

Mitotic figures were generally found in many of the control and treated animals in a low number, (less than 5). An increased amount of mitotic figures (more than 5) was recorded for 5/8 high- and mid-dose females each and 1/8 low dose, 3/8 mid-dose, and 1/8 high-dose males after a 1-week treatment. For the 3-day treatment groups, an increase in mitotic figures was noted for 3/8 low and 5/8 mid-dose males and for all mid- and high-dose females. No increases were seen the high-dose males. The 2-week treatment led to increased mitotic figures in 2/8 mid- and all high-dose males and in 2/8 mid- and 6/8 high-dose females.

4. **Determination of Cell Proliferation (S-Phase Response).** Tables 1B-19 - 1B-22).

The summary tables below show the absolute and relative data for the three different zones in the liver and the mean values for the whole lobule. The relative values (%) are related to the control values (=100). [MRID 45803601, pp 88-91; ATTACHMENT Tables 1B-19 to 1B-22)

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3-day Treatment								
Group	Zone 1		Zone 2		Zone 3		All Zones	
	LI	%	LI	%	LI	%	LI	%
Males								
Control	0.86	100	1.67	100	0.49	100	1.01	100
350 ppm	1.15	134	2.97**	178	0.91	186	1.68**	167
1735 ppm	3.58**	416	5.23**	313	1.67	341	3.49**	347
5210 ppm	1.64*	191	2.28	137	0.69	141	1.54	153
Females								
Control	0.15	100	2.79	100	0.13	100	1.02	100
350 ppm	0.38*	253	2.80	100	0.08	62	1.09	106
1735 ppm	1.08**	720	5.76**	206	0.34	262	2.39**	234
5210 ppm	5.05**	3367	9.35**	335	1.16	892	5.19**	507

LI = Labeling Index.

$$\% = \frac{\text{LI Test}}{\text{LI Control}} \times 100$$

* Statistically significant (WILCOXON Test, two-sided, $p < 0.05$)

** Statistically significant (WILCOXON Test, two-sided, $p < 0.01$)

As shown above for all dose groups after 3 days of treatment, increased cell proliferation (indicated by increased LIs) was observed in at least one of the three zones of the liver. In the females, a clear dose-response relationship was visible for individual zones as well as for all zones combined. Although the response of male livers to the high-dose showed an increase in S-phase synthesis, the response was not dose-related.

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1-week Treatment								
Group	Zone 1		Zone 2		Zone 3		All Zones	
	LI	%	LI	%	LI	%	LI	%
Males								
Control	0.86	100	1.67	100	0.49	100	1.01	100
350 ppm	2.63**	306	7.50**	449	2.08**	424	4.07**	404
1735 ppm	14.03**	1631	15.04**	901	13.21**	2696	14.09**	1400
5210 pp	34.67**	4031	12.44	745	14.72**	3004	20.61**	2047
Females								
Control	0.15	100	2.79	100	0.13	100	1.02	100
350 ppm	0.46**	307	3.31	119	0.16	123	1.31*	128
1735 ppm	1.26**	840	8.77**	314	1.00**	769	3.68**	359
5210 ppm	4.25**	2833	12.57**	451	1.76	1354	6.199**	605

* Statistically significant (WILCOXON Test, two-sided, $p \leq 0.05$)** Statistically significant (WILCOXON Test, two sided, $p \leq 0.01$)

In all dose groups, an increase of cell proliferation was observed at least in one of the three zones of the liver after 1 week of treatment. In the males as well as in the females, a clear dose-response relationship was obtained (see table shown above). A powerful response was induced in all zones of the male liver at 1 week as compared to 3 days of treatment. For example, % LI for all zones of the male liver at 1 week as compared to 3 days of treatment. For example, % LI for all zones was increased 153% after 3 days of treatment versus 2047% after 1 week (a 13-fold increase). For females, the increase between 3 days and 1 week was generally ≤ 1.5 -fold.

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2-week Treatment								
Group	Zone 1		Zone 2		Zone 3		All Zones	
	LI	%	LI	%	LI	%	LI	%
Males								
Control	1.18	100	2.31	100	0.75	100	1.41	100
350 ppm	1.80	153	2.77	120	1.79*	239	2.12	150
1735 ppm	4.14**	351	2.57	111	2.86**	381	3.19**	226
5210 ppm	26.87**	2277	11.54**	500	1.22*	163	13.21**	935
Females								
Control	0.42	100	4.18	100	0.11	100	1.57	100
350 ppm	0.71	169	3.39*	81	0.08	73	1.39	89
1735 ppm	1.78**	424	4.89	117	1.33**	1209	2.67	170
5210 ppm	7.54**	1795	1.47**	35	1.41*	373	3.14	200

* Statistically significant (WILCOXON Test, two-sided, $p \leq 0.05$)** Statistically significant (WILCOXON Test, two-sided $p \leq 0.01$)

After 2 weeks of treatment at the high- and mid-dose groups, an increased cell proliferation was observed at least in one of the three zones of the liver. In both males and females, a clear dose-response relationship was visible for all dose groups. Only in the low dose group of males was a significant increase in the LI seen. As compared to the earlier % LIs increases, values generally declined for all doses in both males and females.

5. **Determination of Apoptosis (TUNEL Stain)** (MRID 45803601, pp 92-96; ATTACHMENT Tables 1B-23 - 1B-26)

The summary tables below show the total counts of apoptotic bodies in the livers of control and treated animals.

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3-Day Treatment				
Group	Zone 1	Zone 2	Zone 3	Zone 1+2+3
Total Counts (Males)				
Control	0	1	2	3
350 ppm	0	1	1	2
1735 ppm	0	0	5	5
5210 ppm	0	2	1	3
Total Counts (Females)				
Control	2	3	2	7
350 ppm	0	3	0	3
1735 ppm	0	1	1	2
5210 ppm	0	4	6	10

1-week Treatment				
Group	Zone 1	Zone 2	Zone 3	Zone 1+2 + 3
Total Counts (Males)				
Control	0	1	2	3
350 ppm	1	1	1	3
1735 ppm	2	2	3	7
5210 ppm	0	1	1	2
Total Counts (Females)				
Control	2	3	2	7
350 ppm	4	2	2	8
1735 ppm	3	3	4	10
5210 ppm	1	4	8	13

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2-week Treatment				
Group	Zone 1	Zone 2	Zone 3	Zone 1+2+3
Total Counts (Males)				
Control	0	4	1	5
350 ppm	0	0	1	1
1735 ppm	3	2	2	7
5210 ppm	10*	31**	108**	149**
Total Counts (Females)				
Control	0	1	2	3
350 ppm	1	0	1	2
1735 ppm	0	1	0	1
5210 ppm	5	34	80	119

* Statistically significant (Wilcoxon test, two-sided, $p \leq 0.05$)

** Statistically significant (Wilcoxon test, two-sided, $p \leq 0.01$)

Only single apoptotic cells were found in the control, the low and mid-dose groups. No biologically relevant increases in apoptotic cells were noted in either sex of all treatment groups. After 2 weeks of treatment, however, a significant increase was noted for the high-dose males. No statistical significance was calculated for the females since only 2 of the 8 test females had excessively high counts that contributed to the elevated counts for Zones 2 and 3 and the combined total count. The remaining females had counts ranging from 0-6 for individual zones.

III INVESTIGATORS' CONCLUSIONS AND DISCUSSION

At the high dose (5210 ppm, *i.e.*, 2400 ppm a.i.), Blazer Technical produced slight but significant ($p \leq 0.01$) body weight decreases in males (only) at all time periods (3-day, 1-week and 4-week). However, our reviewers noted that significant findings occurred sporadically in all three subsets of males. Consequently, we concluded that there was no clear or consistent effect on male body weight.

In all dose groups (350, 1735 and 5210 ppm, Blazer Technical; representing 160, 800, and 2400 ppm a.i.) and after all three treatment periods, increased BrdU LIs, indicative of cell proliferation were induced. Increases for all zones of the treated liver lobule ranged from 3.5-fold for males to 5.2-fold for females (the 3-day treatment); 20-fold for males and 6.2-fold for females (1-week treatment); 9.4-fold for males and 2-fold for females (2-week treatment). The

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DNA SYNTHESIS (S-PHASE RESPONSE)/CELL PROLIFERATION

most prominent induction of cell proliferation with the most distinct dose response relationship was observed for both sexes after the 1-week treatment. After the 2-week treatment, apoptotic cells were found in the high-dose males but only in single high-dose females. [This increase was considered by the investigators to be a secondary, reactive response in the induction of cell proliferation ("a down-regulating mechanism"), which our reviewers noted, would agree with the clearly lower LIs after the 2-week treatment period when compared with the high LIs measured after the 1-week treatment period.

Dose-dependent increases in the liver weights and histopathological findings of panlobular and centrilobular hypertrophy with enhanced cytoplasmic eosinophilia in both sexes correlated favorably with findings described and analyzed in a previous electron microscopic study on liver peroxisomes (MRID 45693401; BASF Project No. 99C0287/98154), which reported a dose-dependent increase in the amount of peroxisomes and peroxisomal areas after 4 weeks of treatment with the same doses as used in this study. The increased number of mitotic figures, although less uniformly observed, correlated with the induction of cell proliferation. The report of the investigators also indicated that the high-dose of Blazer Technical (5210 ppm) produced an increase in degenerative findings ("single cell necrosis") including hyaline droplets and apoptotic cells, respectively. The 2-week treatment with the test substance also produced an inflammatory response for the male group, with single cell necrosis more pronounced compared to the shorter treatment periods. The investigators interpret these findings as signs of cytotoxicity on hepatocytes.

The investigators concluded that the dietary administration of Blazer technical (of purity 46.1% acifluorfen sodium) resulted in dose-dependent induction of cell proliferation (S-phase response) in mice treated at levels of 350, 1735 and 5210 ppm (160, 800 and 2400 ppm a.i.), an effect that was most pronounced after the 1-week treatment.

IV REVIEWERS' DISCUSSION AND CONCLUSIONS

The EPA reviewers agree with the investigators' conclusion that administration of Blazer Technical (46.1% acifluorfen sodium) in the diet for 3 days, 1-week and 2-week treatments at levels (350, 1735 and 5210; 160, 800 and 2400 ppm a.i.) induced dose-related increases in liver weights (generally both absolute and relative). Findings of increased panlobular hypertrophy of hepatocytes were also seen in both males and females exposed to 714/845 a.i. mg/kg/day, respectively, on all treatment schedules (3-day, 1- and 2-week). Liver hypertrophy was most pronounced in the males and peaked after 1 week of treatment. By 2 weeks, an increase in single cell necrosis and apoptotic cells was observed primarily in high-dose males. This finding, suggesting cytotoxicity to the target organ, indicates that dosing was adequate to assess cell proliferation in the liver. In addition, the test material produced a dose-dependent and significant induction of BrdU labeling in the liver, indicative of cell proliferation (S-phase response), in all dose groups. The most pronounced effect was seen after 1 week of treatment, with a dose-related increase in cell proliferation (in males) and liver weight (in both males and females). Overall, the data from this study show that Blazer Technical, containing 46 % Na acifluorfen as the a. i., induced a dose-related increase in liver weights, liver hypertrophy and high BrdU

ACIFLUORFEN, SODIUM

DNA SYNTHESIS (S-PHASE RESPONSE)/CELL PROLIFERATION

labeling indices. The most prominent induction of all three parameters for both sexes occurred after 1 week of treatment.

V. STUDY DEFICIENCIES:

No serious major deficiencies were found which would alter the conclusions of the investigators.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

BODY WEIGHT

Print Date: 04-mar-2002
 Print Time: 10:58:40
 Table : IA
 Page : 13

	Body Weight g Day 0	Body Weight g Day 4	Body Weight g Day 7
Male. group 00 0 ppm			
Mean	28.6	30.7	30.9
SD	1.7	1.4	1.4
N	8	8	8
%dev			
Male. group 01 350 ppm			
Mean	27.7	30.0	30.4
SD	1.2	1.3	1.2
N	8	8	8
%dev	-3.2	-2.2	-1.6
Male. group 02 1735 ppm			
Mean	28.2	30.7	31.2
SD	0.9	0.9	0.7
N	8	8	8
%dev	-1.5	0.2	1.0
Male. group 03 5210 ppm			
Mean	28.1	29.0*	29.8
SD	1.3	1.1	1.0
N	8	8	8
%dev	-1.9	-5.4	-3.4

Key: D - Dunnett's test. Two-sided. * - 0.050. ** - 0.010
 Experimental Unit - Animal

BODY WEIGHT

Print Date: 04-Mar-2002
Print Time: 11:24:50
Table : IA
Page : 14

	Body Weight Day 4 D	Body Weight Day 7 D
Male, group 00 0 ppm		
Mean	30.7	30.9
SD	1.4	1.4
N	8	8
%dev		
Male, group 11 350 ppm		
Mean	30.0	31.0
SD	1.3	1.4
N	8	8
%dev	-2.3	0.4
Male, group 12 1735 ppm		
Mean	30.4	31.4
SD	0.5	0.5
N	8	8
%dev	-1.1	1.6
Male, group 13 5210 ppm		
Mean	30.2	28.3**
SD	1.0	1.1
N	8	8
%dev	-1.5	-8.4

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

BODY WEIGHT

Print Date: 12-Mar-2002
 Print Time: 11:52:03
 Table : IA
 Page : 15

	Body Weight g Day 0	Body Weight g Day 7	Body Weight g Day 14
Male, group 20 0 ppm			
Mean	29.4	29.1	31.4
SD	1.1	1.2	0.8
N	8	8	8
%dev			
Male, group 21 350 ppm			
Mean	30.1	30.2	31.6
SD	1.4	1.1	0.7
N	8	8	8
%dev	2.3	3.9	0.8
Male, group 22 1735 ppm			
Mean	29.8	30.7*	32.5
SD	1.0	1.2	0.9
N	8	8	8
%dev	1.3	5.6	3.5
Male, group 23 5210 ppm			
Mean	29.2	29.0	29.0**
SD	1.1	1.6	1.8
N	8	8	8
%dev	-0.5	-0.1	-7.6

Key: D = Dunnett's test, Two-sided. * = 0.050, ** = 0.010
 Experimental Unit - Animal

BODY WEIGHT

Print Date: 04-Mar-2002
Print Time: 11:01:35
Table : IA
Page : 16

	Body Weight g Day 0	Body Weight g Day 4	Body Weight g Day 7
Female, group 00 0 ppm			
Mean	24.0	26.5	26.7
SD	1.2	0.9	0.9
N	8	8	4
%dev			
Female, group 01 350 ppm			
Mean	24.1	26.6	27.2
SD	1.1	1.0	1.2
N	8	8	4
%dev	0.5	0.7	2.1
Female, group 02 1735 ppm			
Mean	24.3	27.1	27.9
SD	0.7	1.0	1.0
N	8	8	4
%dev	1.4	2.5	4.6
Female, group 03 5210 ppm			
Mean	23.7	26.2	26.9
SD	0.9	1.5	1.3
N	8	8	4
%dev	-1.3	-1.1	1.0

Key: D = Dunnett's test. Two-sided. * = 0.050. ** = 0.010
Experimental Unit = Animal

BODY WEIGHT

Print Date: 12-Mar-2002
 Print Time: 12:12:08
 Table : IA
 Page : 17

	Body Weight g Day 4 D	Body Weight g Day 7 D
Female, group 00 0 ppm		
Mean	26.5	26.5
SD	0.9	0.9
N	8	8
%dev		
Female, group 11 350 ppm		
Mean	26.8	26.8
SD	1.0	1.3
N	8	8
%dev	1.1	1.0
Female, group 12 1735 ppm		
Mean	26.6	27.2
SD	0.8	0.7
N	8	8
%dev	0.4	2.5
Female, group 13 5210 ppm		
Mean	26.2	26.1
SD	1.8	1.3
N	8	8
%dev	-1.1	-1.5

Key: D - Dunnett's test, Two-sided. * - 0.050, ** - 0.010
 Experimental Unit - Animal

BODY WEIGHT

Print Date: 12-Mar-2002
 Print Time: 11:55:08
 Table : 1A
 Page : 18

	Body Weight g Day 0 D	Body Weight g Day 7 D	Body Weight g Day 14 D
Female. group 20 0 ppm			
Mean	22.6	23.5	26.6
SD	1.5	1.7	1.5
N	8	8	8
%dev			
Female. group 21 350 ppm			
Mean	23.0	23.7	26.8
SD	1.0	1.0	0.9
N	8	8	8
%dev	1.4	0.7	0.8
Female. group 22 1735 ppm			
Mean	23.2	23.9	26.8
SD	1.2	1.3	1.2
N	8	8	8
%dev	2.5	1.4	0.8
Female. group 23 5210 ppm			
Mean	23.4	24.2	26.3
SD	0.9	1.3	1.1
N	8	8	8
%dev	3.6	3.0	-1.1

Key: D = Dunnett's test, Two-sided. * = 0.050, ** = 0.010
 Experimental Unit - Animal

BASF - DATATOX-F1 R11

Study: 99C0287/98153

SUBSTANCE INTAKE

Print Date: 18-Jun-2002
Print Time: 07:24:16
Table : IA
Page : 19

Subst.intake
mg/kg Bw
Day 7

Male. group 01 350 ppm

Mean	83.8
SD	12.0
N	8

Male. group 02 1735 ppm

Mean	499.6
SD	135.9
N	8

Male. group 03 5210 ppm

Mean	1652.4
SD	478.3
N	8

Key: Experimental Unit - Animal

BASF - DATATOX-F1 R11

Study: 99C0287/98153

SUBSTANCE INTAKE

Print Date: 18-Jun-2002
Print Time: 07:22:27
Table : 1A
Page : 20

Subst.intake
mg/kg Bw
Day 7

Male, group 11 350 ppm

Mean	82.9
SD	28.3
N	8

Male, group 12 1735 ppm

Mean	527.0
SD	308.2
N	8

Male, group 13 5210 ppm

Mean	1498.6
SD	778.0
N	8

Key: Experimental Unit - Animal

BASF - DATATOX-F1 R11

Study: 99C0287/98153

SUBSTANCE INTAKE

Print Date: 18-Jun-2002
Print Time: 07:20:51
Table : IA
Page : 21

	Subst.intake mg/kg Bw Day 7	Subst.intake mg/kg Bw Day 14
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Male, group 21 350 ppm

Mean	88.7	92.4
SD	29.1	34.8
N	8	8

Male, group 22 1735 ppm

Mean	413.8	488.2
SD	161.0	178.8
N	8	8

Male, group 23 5210 ppm

Mean	1434.6	1552.8
SD	256.2	416.5
N	8	8

Key: Experimental Unit - Animal

BASF - DATATOX-F1 R11

Study: 99C0287/98153

SUBSTANCE INTAKE

Print Date: 18-Jun-2002
Print Time: 07:29:13
Table : 1A
Page : 22

Subst.intake
mg/kg Bw
Day 7

Female, group 01 350 ppm

Mean	112.0
SD	24.2
N	8

Female, group 02 1735 ppm

Mean	600.4
SD	143.6
N	8

Female, group 03 5210 ppm

Mean	1595.1
SD	389.4
N	8

Key: Experimental Unit - Animal

BASF - DATATOX-F1 R11

Study: 99C0287/98153

SUBSTANCE INTAKE

Print Date: 18-Jun-2002
Print Time: 07:23:16
Table : 1A
Page : 23

Subst.intake
mg/kg Bw
Day 7

Female, group 11 350 ppm

Mean	121.6
SD	46.3
N	8

Female, group 12 1735 ppm

Mean	641.9
SD	184.1
N	8

Female, group 13 5210 ppm

Mean	1802.2
SD	606.4
N	8

Key: Experimental Unit - Animal

BASF - DATATOX-F1 R11

Study: 99C0287/98153

SUBSTANCE INTAKE

Print Date: 18-Jun-2002
Print Time: 07:21:18
Table : IA
Page : 24

	Subst.intake mg/kg Bw Day 7	Subst.intake mg/kg Bw Day 14
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Female, group 21 350 ppm

Mean	131.0	107.6
SD	52.3	45.1
N	8	8

Female, group 22 1735 ppm

Mean	603.3	647.1
SD	130.3	191.0
N	8	8

Female, group 23 5210 ppm

Mean	2088.2	2109.2
SD	394.8	564.5
N	8	8

Key: Experimental Unit - Animal

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

ABSOLUTE WEIGHTS - MEAN VALUES (MALE, 3-DAY TREATMENT)

Sacrifice group			F1			
Sex			M			
Dose group			00	11	12	13
Terminal body weight	g	M	29.963	29.288	29.363	26.325**
		SD	1.334	1.146	0.566	0.8
		n	8	8	8	8
Liver	g	M	1.509	1.454	1.719**	1.591
		SD	0.09	0.046	0.077	0.106
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

ABSOLUTE WEIGHTS - MEAN VALUES (FEMALE, 3-DAY TREATMENT)

Sacrifice group			F1			
Sex			F			
Dose group			00	11	12	13
Terminal body weight	g	M	25.9	25.738	25.963	24.9
		SD	0.84	1.058	0.717	1.343
		n	8	8	8	8
Liver	g	M	1.311	1.328	1.529**	1.918**
		SD	0.093	0.096	0.081	0.129
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

RELATIVE WEIGHTS - MEAN VALUES (MALE, 3-DAY TREATMENT)

Sacrifice group		F1			
Sex		M			
Dose group		00 11 12 13			
Terminal body weight	%	M	100.0	100.0	100.0
		n	8	8	8
Liver	%	M	5.036	4.976	5.859**
		SD	0.308	0.192	0.231
		n	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Ctrl Mice

acopat system

RELATIVE WEIGHTS - MEAN VALUES (FEMALE, 3-DAY TREATMENT)

Sacrifice group			F1			
Sex			F			
Dose group			00	11	12	13
Terminal body weight	‡	M	100.0	100.0	100.0	100.0
		n	8	8	8	8
Liver	‡	M	5.064	5.156	5.887**	7.697**
		SD	0.227	0.228	0.271	0.269
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

ABSOLUTE WEIGHTS - MEAN VALUES (MALE, 1-WEEK TREATMENT)

Sacrifice group			F1			
Sex			M			
Dose group			00	01	02	03
Terminal body weight	g	M	29.963	29.25	29.8	27.9 **
		SD	1.334	1.032	0.719	1.034
		n	8	8	8	8
Liver	g	M	1.509	1.578	1.953**	2.113**
		SD	0.09	0.072	0.04	0.461
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

ABSOLUTE WEIGHTS - MEAN VALUES (FEMALE, 1-WEEK TREATMENT)

Sacrifice group			F1			
Sex			F			
Dose group			00	01	02	03
Terminal body weight	g	M	25.9	26.2	26.613	25.55
		SD	0.84	0.959	0.966	1.233
		n	8	8	8	8
Liver	g	M	1.311	1.363	1.833**	2.365**
		SD	0.093	0.054	0.112	0.191
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

RELATIVE WEIGHTS - MEAN VALUES (MALE, 1-WEEK TREATMENT)

Sacrifice group			F1			
Sex			M			
Dose group			00	01	02	03
Terminal body weight	†	M	100.0	100.0	100.0	100.0
		n	8	8	8	8
Liver	†	M	5.036	5.392*	6.556**	7.56 *
		SD	0.308	0.272	0.16	1.592
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

RELATIVE WEIGHTS - MEAN VALUES (FEMALE, 1-WEEK TREATMENT)

Sacrifice group			F1			
Sex			F			
Dose group			00	01	02	03
Terminal body weight	%	M	100.0	100.0	100.0	100.0
		n	8	8	8	8
Liver	%	M	5.064	5.202	6.881**	9.256**
		SD	0.227	0.111	0.267	0.53
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

ABSOLUTE WEIGHTS - MEAN VALUES (MALE, 2-WEEK TREATMENT)

Sacrifice group			F1			
Sex			M			
Dose group			20	21	22	23
Terminal body weight	g	M	30.6	30.525	31.263	27.338**
		SD	0.784	0.654	0.877	1.537
		n	8	8	8	8
Liver	g	M	1.513	1.629**	2.125**	1.898**
		SD	0.047	0.048	0.072	0.15
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Ctrl Mice

acopat system

ABSOLUTE WEIGHTS - MEAN VALUES (FEMALE, 2-WEEK TREATMENT)

Sacrifice group			F1			
Sex			F			
Dose group			20	21	22	23
Terminal body weight	g	M	25.625	25.563	25.45	24.838
		SD	1.451	1.036	1.163	1.171
		n	8	8	8	8
Liver	g	M	1.299	1.314	1.615**	2.185**
		SD	0.105	0.086	0.073	0.058
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

RELATIVE WEIGHTS - MEAN VALUES (MALE, 2-WEEK TREATMENT)

Sacrifice group			F1			
Sex			M			
Dose group			20	21	22	23
Terminal body weight	%	M	100.0	100.0	100.0	100.0
		n	8	8	8	8
Liver	%	M	4.944	5.335**	6.797**	6.935**
		SD	0.118	0.147	0.216	0.34
		n	8	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Cr1 Mice

acopat system

RELATIVE WEIGHTS - MEAN VALUES (FEMALE, 2-WEEK TREATMENT)

Sacrifice group		F1			
Sex		F			
Dose group		20	21	22	23
Terminal body weight	%	M	100.0	100.0	100.0
		n	8	8	8
Liver	%	M	5.065	5.14	6.347**
		SD	0.23	0.23	0.21
		n	8	8	8

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

BASF

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PATHOLOGY REPORT99C0287/98153S-Phase Response in the Liver of B6C3F1/Oct/14/2002 WEKACrl Miceacopat systemINCIDENCE OF GROSS LESIONS (3-DAY TREATMENT)

Sacrifice group	F1							
	M				F			
Dose group	00	11	12	13	00	11	12	13
Animals in selected Group	8	8	8	8	8	8	8	8
NAD	8	8	8	8	8	7	8	8
Ovaries
- Discoloration	1	.	.

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Cr1 Mice

acopat system

INCIDENCE OF GROSS LESIONS (1-WEEK TREATMENT)

Sacrifice group	F1				F			
Sex	M				F			
Dose group	00	01	02	03	00	01	02	03
Animals in selected Group	8	8	8	8	8	8	8	8
NAD	8	8	8	8	8	8	8	.
Liver
- Enlarged	8
- Focus	1
Pancreas
- Cyst	1

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

INCIDENCE OF GROSS LESIONS (2-WEEK TREATMENT)

Sacrifice group	F1				F			
Sex	M							
Dose group	20	21	22	23	20	21	22	23
Animals in selected Group	8	8	8	8	8	8	8	8
NAD	8	8	8	8	8	7	8	1
Liver
- Enlarged	7
Kidneys
- Retraction	1	.	.

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

INCIDENCE AND GRADINGS OF LIVER FINDINGS (3-DAY TREATMENT)

Sacrifice group	F1				F			
Sex	M				F			
Dose group	00	11	12	13	00	11	12	13
Animals in selected Group	8	8	8	8	8	8	8	8
Liver	8	8	8	8	8	8	8	8
- Granuloma(s), Kupff.	3	2	3	2	6	7	5	5
. 1.	3	2	3	2	6	7	5	5
- Hypertrophy, central	.	6	8	.	.	1	8	.
. 1.	.	5	.	.	.	1	.	.
. 2.	.	1	5
. 3.	.	.	3	.	.	.	8	.
- Hypertrophy, diffuse	.	.	.	8	.	.	.	8
. 3.	.	.	.	8	.	.	.	4
. 4.	4
- Increased mitosis	.	3	5	.	.	.	8	8
. 1.	.	.	3	.	.	.	5	.
. 2.	.	2	2	1
. 3.	.	1	1	.	.	.	1	4
. 4.	.	.	1	3
- Fatty change, cent.	.	.	1
. 1.	.	.	1
- Single cell necrosis	1
. 1.	1

BASF

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

INCIDENCE AND GRADINGS OF LIVER FINDINGS (1-WEEK TREATMENT)

Sacrifice group	F1				F			
Sex	M				F			
Dose group	00	01	02	03	00	01	02	03
Animals in selected Group	8	8	8	8	8	8	8	8
Liver	8	8	8	8	8	8	8	8
- Granuloma(s), Kupff.	3	2	4	4	6	7	6	1
. 1.	3	2	3	4	6	7	6	1
. 2.	.	.	1
- Hypertrophy, central	.	7	8	.	.	.	8	.
. 1.	.	6
. 2.	.	1	1	.	.	.	5	.
. 3.	.	.	7	.	.	.	3	.
- Hypertrophy, diffuse	.	.	.	8	.	.	.	8
. 3.	.	.	.	8
. 4.	8
- Increased mitosis	.	1	3	1	.	.	5	5
. 1.	.	1	3	1	.	.	4	4
. 2.	1	1
- Fatty change, cent.	.	.	.	1
. 1.	.	.	.	1
- Single cell necrosis	.	.	.	2	.	.	.	3
. 1.	.	.	.	2	.	.	.	3
- Focal necrosis	1
. P.	1

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PATHOLOGY REPORT

99C0287/98153

S-Phase Response in the Liver of B6C3F1/

Oct/14/2002 WEKA

Crl Mice

acopat system

INCIDENCE AND GRADINGS OF LIVER FINDINGS (2-WEEK TREATMENT)

Sacrifice group	F1				F			
Sex	M				F			
Dose group	20	21	22	23	20	21	22	23
Animals in selected Group	8	8	8	8	8	8	8	8
Liver	8	8	8	8	8	8	8	8
- Granuloma(s), Kupff.	3	4	2	1	6	7	6	4
. 1.	3	4	1	1	6	7	6	4
. 2.	.	.	1
- Hypertrophy, central	.	8	8	.	.	2	8	.
. 1.	.	8	.	.	.	1	.	.
. 2.	1	1	.
. 3.	.	.	8	.	.	.	7	.
- Hypertrophy, diffuse	.	.	.	8	.	.	.	8
. 3.	.	.	.	8
. 4.	8
- Increased mitosis	.	.	2	8	.	.	2	6
. 1.	.	.	1	2	.	.	2	4
. 2.	.	.	1	2
. 3.	.	.	.	1
. 4.	.	.	.	5
- Single cell necrosis	.	.	.	8	.	.	.	8
. 1.	1
. 2.	.	.	.	2	.	.	.	7
. 3.	.	.	.	6
- Infiltrates, mononuc.	.	.	.	8
. 1.	.	.	.	1
. 2.	.	.	.	7

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PATHOLOGY REPORT

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99C0287/98153

S-Phase-Response in the Liver of B6C6F1/Crl Mice

Sept/24/2002 WEKA

BrdU – Labeling Indices (LI) of Liver – Mean Values (Male)

3 – day treatment:

		Zone 1	Zone 2	Zone 3	All Zones
Dose (ppm)		LI	LI	LI	LI
350	M	1.15	2.97 **	0.91 **	1.68 **
	SD	0.48	0.94	0.31	0.50
	n	8	8	8	8
1,735	M	3.58 **	5.23 **	1.67 **	3.49 **
	SD	1.23	1.83	0.84	1.24
	n	8	8	8	8
5,210	M	1.64 *	2.28	0.69	1.54
	SD	0.91	1.05	0.48	0.75
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

1 – week treatment:

		Zone 1	Zone 2	Zone 3	All Zones
Dose (ppm)		LI	LI	LI	LI
Control	M	0.86	1.67	0.49	1.01
	SD	0.44	0.57	0.14	0.30
	n	8	8	8	8
350	M	2.63 **	7.50 **	2.08 **	4.07 **
	SD	1.43	3.57	0.76	1.86
	n	8	8	8	8
1,735	M	14.03 **	15.04 **	13.21 **	14.09 **
	SD	2.69	2.69	3.31	2.69
	n	8	8	8	8
5,210	M	34.67 **	12.44 **	14.72 **	20.61 **
	SD	8.89	2.41	4.13	3.57
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

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PATHOLOGY REPORT

IB- 20
99C0287/98153

S-Phase-Response in the Liver of B6C6F1/Crl Mice

Sept/24/2002 WEKA

BrdU – Labeling Indices (LI) of Liver – Mean Values (Male)

2 – week treatment:

		Zone 1	Zone 2	Zone 3	All Zones
Dose (ppm)		LI	LI	LI	LI
Control	M	1.18	2.31	0.75	1.41
	SD	0.47	0.77	0.73	0.53
	n	8	8	8	8
350	M	1.80	2.77	1.79 *	2.12
	SD	0.93	1.44	1.05	0.96
	n	8	8	8	8
1,735	M	4.14 **	2.57	2.86 **	3.19 **
	SD	2.00	0.96	1.47	1.18
	n	8	8	8	8
5,210	M	26.87 **	11.54 **	1.22 *	13.21 **
	SD	5.63	5.35	0.53	3.57
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

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PATHOLOGY REPORT**
S-Phase-Response in the Liver of B6C3F1/Crl Mice
**IB- 21
99C0287/98153
Sept/24/2002 WEKA**
BrdU – Labeling Indices (LI) of Liver – Mean Values (Female)
3 – day treatment:

		Zone 1	Zone 2	Zone 3	All Zones
Dose (ppm)		LI	LI	LI	LI
350	M	0,38 *	2,80	0,08	1,09
	SD	0,26	0,93	0,08	0,39
	n	8	8	8	8
1,735	M	1,08 **	5,76 **	0,34	2,39 **
	SD	0,51	1,40	0,29	0,70
	n	8	8	8	8
5,210	M	5,05 **	9,35 **	1,16 **	5,19 **
	SD	1,33	0,93	0,37	0,70
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

1 – week treatment:

		Zone 1	Zone 2	Zone 3	All Zones
Dose (ppm)		LI	LI	LI	LI
Control	M	0,15	2,79	0,13	1,02
	SD	0,14	0,50	0,16	0,24
	n	8	8	8	8
350	M	0,46 **	3,31	0,16	1,31 *
	SD	0,27	0,81	0,09	0,31
	n	8	8	8	8
1,735	M	1,26 **	8,77 **	1,00 **	3,68 **
	SD	0,19	1,71	0,42	0,70
	n	8	8	8	8
5,210	M	4,25 **	12,57 **	1,76 **	6,19 **
	SD	0,94	3,05	0,57	1,37
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

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PATHOLOGY REPORT

S-Phase-Response in the Liver of B6C3F1/Crl Mice

IB- 22
99C0287/98153
Sept/24/2002 WEKA

BrdU – Labeling Indices (LI) of Liver – Mean Values (Female)

2 – week treatment:

		Zone 1	Zone 2	Zone 3	All Zones
Dose (ppm)		LI	LI	LI	LI
Control	M	0,42	4,18	0,11	1,57
	SD	0,13	0,45	0,11	0,18
	n	8	8	8	8
350	M	0,71	3,39 *	0,08	1,39
	SD	0,48	0,88	0,11	0,41
	n	8	8	8	8
1,735	M	1,78 **	4,89	1,33 **	2,67 *
	SD	0,56	1,51	0,42	0,64
	n	8	8	8	8
5,210	M	7,54 **	1,47 **	0,41 *	3,14 **
	SD	0,69	0,40	0,27	0,26
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

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 PATHOLOGY REPORT
 S-Phase-Response in the Liver of B6C6F1/Crl Mice

IB- 23
 99C0287/98153
 Sept/24/2002 WEKA

TUNEL – Total Counts (Σ) of Liver – Mean Values (Male)

3 – day treatment:

Dose (ppm)		Zone 1	Zone 2	Zone 3	All Zones
350	Σ	0	1	1	2
	SD	0,00	0,35	0,35	0,46
	n	8	8	8	8
1,735	Σ	0	0	5	5
	SD	0,00	0,00	0,92	0,92
	n	8	8	8	8
5,210	Σ	0	2	1	3
	SD	0,00	0,46	0,35	0,74
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

1 – week treatment:

Dose (ppm)		Zone 1	Zone 2	Zone 3	All Zones
Control	Σ	0	1	2	3
	SD	0,00	0,35	0,71	0,74
	n	8	8	8	8
350	Σ	1	1	1	3
	SD	0,35	0,35	0,35	0,74
	n	8	8	8	8
1,735	Σ	2	2	3	7
	SD	0,71	0,46	0,74	1,25
	n	8	8	8	8
5,210	Σ	0	1	1	2
	SD	0,00	0,35	0,35	0,46
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

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 PATHOLOGY REPORT
 S-Phase-Response in the Liver of B6C6F1/Crl Mice

IB- 24
 99C0287/98153
 Sept/24/2002 WEKA

TUNEL – Total Counts (Σ) of Liver – Mean Values (Male)

2 – week treatment:

Dose (ppm)		Zone 1	Zone 2	Zone 3	All Zones
Control	Σ	0	4	1	5
	SD	0,00	0,93	0,35	1,19
	n	8	8	8	8
350	Σ	0	0	1	1
	SD	0,00	0,00	0,35	0,35
	n	8	8	8	8
1,735	Σ	3	2	2	7
	SD	0,74	0,46	0,71	1,13
	n	8	8	8	8
5,210	Σ	10 *	31 **	108 **	149 **
	SD	1,49	3,09	10,20	13,18
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)

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S-Phase-Response in the Liver of B6C6F1/Crl Mice

Sept/24/2002 WEKA

TUNEL – Total Counts (Σ) of Liver – Mean Values (Female)

3 – day treatment:

Dose (ppm)		Zone 1	Zone 2	Zone 3	All Zones
350	Σ	0	3	0	3
	SD	0,00	0,52	0,00	0,52
	n	8	8	8	8
1,735	Σ	0	1	1	2
	SD	0,00	0,35	0,35	0,46
	n	8	8	8	8
5,210	Σ	0	4	6	10
	SD	0,00	0,93	1,39	1,39
	n	8	8	8	8

* : $p \leq 0.05$ ** : $p \leq 0.01$: Wilcoxon-test (two-sided)

1 – week treatment:

Dose (ppm)		Zone 1	Zone 2	Zone 3	All Zones
Control	Σ	2	3	2	7
	SD	0,46	0,52	0,46	0,84
	n	8	8	8	8
350	Σ	4	2	2	8
	SD	0,54	0,46	0,46	0,93
	n	8	8	8	8
1,735	Σ	3	3	4	10
	SD	0,74	0,52	0,54	1,58
	n	8	8	8	8
5,210	Σ	1	4	8	13
	SD	0,35	0,76	0,93	1,19
	n	8	8	8	8

* : $p \leq 0.05$ ** : $p \leq 0.01$: Wilcoxon-test (two-sided)

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PATHOLOGY REPORT

IB- 26

99C0287/98153

S-Phase-Response in the Liver of B6C6F1/Crl Mice

Sept/24/2002 WEKA

TUNEL – Total Counts (Σ) of Liver – Mean Values (Female)

2 – week treatment:

Dose (ppm)		Zone 1	Zone 2	Zone 3	All Zones
Control	Σ	0	1	2	3
	SD	0,00	0,35	0,46	0,52
	n	8	8	8	8
350	Σ	1	0	1	2
	SD	0,35	0,00	0,35	0,71
	n	8	8	8	8
1,735	Σ	0	1	0	1
	SD	0,00	0,35	0,00	0,35
	n	8	8	8	8
5,210	Σ	5	34	80	119
	SD	1,06	8,33	17,79	26,92
	n	8	8	8	8

* : $p \leq 0.05$

** : $p \leq 0.01$: Wilcoxon-test (two-sided)



13544

062708

Chemical: Benzoic acid, 5-(2-chloro-4-(trifluorome

PC Code: 114402

HED File Code 11000 Chemistry Reviews

Memo Date: 05/13/2003

File ID: TX051328

Accession Number: 412-03-0114

HED Records Reference Center

07/29/2003